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REPORTS

FROM

THE LABORATORY

OF THE

Royal College of Physicians

EDINBURGH

EDITED BY

Sir JOHN BATTY TUKE, M.D.

AND

JAMES RITCHIE, M.D.

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PREFACE

THE present volume of Reports contains the contributions of workers up to the end of 1910.

It is proper here to acknowledge the continued assistance received from the Carnegie Trust by those engaged in research in the Laboratory. Not only have grants been freely given to workers, but in 1907, the Trust voted a sum of £600 for improving the scientific equipment of the Laboratory, and the provision thus made has in many ways contributed to the efficiency of the work done.

In 1908, the Laboratory Committee set apart a special room for the investigation of neurological problems, and the present volume gives evidence of the advantage derived from the facilities thus offered.

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ON THE ORIGIN OF THE FACIAL NERVE.

BY

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EDINBURGH.

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ON THE ORIGIN OF THE FACIAL NERVE.

By ALEXANDER BRUCE, M.D., F.R.C.P.E., Physician to the Royal Infirmary, Edinburgh; and J. H. HARVEY PIRIE, M.D., B.Sc., Clinical Tutor in the Royal Infirmary, Edinburgh, Assistant to the Professor of Medical Jurisprudence, University of Edinburgh.

THE origin of the facial nerve has been the subject of much investigation and of many controversies. It is long since its alleged connection with the abducens nucleus has been disproved by Van Gudden and Gowers (1), and since Lockhart Clarke's (2) view as to the inferior facial nucleus has been set aside; but a number of other questions, which are of interest and importance from both the anatomical and the clinical standpoint, still remain to be settled. The object of the present paper is to endeavour to answer some of these, such as, for instance :—

(1) Whether all its fibres arise from the well-known main nucleus in the pons?

(2) Whether any of them have a crossed origin from the corresponding nucleus of the opposite side?

(3) Whether there is a separate nucleus for the muscles of the upper face, viz., the orbicularis palpebrarum

frontalis, corrugator supercilii, and, if so, where is it situated?

(4) Whether there is a separate nucleus for the muscles of the lips, and, if so, where is it situated?

The third and fourth questions have been raised by physicians in order to explain certain clinical phenomena which appear to be incompatible with the assumption that all the muscles of the face could be innervated from a common centre. The fact that in such diseases as bulbar paralysis and amyotrophic lateral sclerosis there is a comparative escape of the muscles of the upper face, while the muscles of the middle and of the lower face are profoundly involved, has suggested the possible existence of a nucleus for the nerves of supply of the upper face independent of the main nucleus of the facial nerve in the pons. On the other hand, the close association which exists between certain movements of the tongue and of the lips has led to the view that there must be a similarly close association of their nuclei of supply. Charcot (3) and Ross (4), following Lockhart Clarke (2), held that the muscles of the lips are innervated from an inferior facial nucleus, situated partly above the hypoglossal nucleus and partly also between it and the ependyma of the fourth ventricle; while Gowers (1) maintains that the centre for the lip muscles is in the upper extremity of the hypoglossal nucleus itself.

A. With regard to the nucleus for the upper facial muscles, Mendel (5), after section of the upper facial nerve in young rabbits and guinea-pigs, found atrophy of the cells in the lower part of the oculo-motor nucleus of the same side, and traced a connection to the facial nerve through the posterior longitudinal fasciculus. Mendel's belief that this group of cells constituted the nucleus for the upper facial nerve, apparently so completely solved the difficulty of the clinician that there has been a tendency to accept its existence as an independent nucleus without further sufficient proof. The remarkable immunity of the upper facial muscles in bulbar paralysis and in amyotrophic sclerosis, even where there is a high degree of paralysis of the lower face, has from the first been difficult to explain, and it has seemed essential to premise the existence of a nucleus altogether independent of the facial nucleus and escaping the degeneration

in which the latter is involved. Mendel's discovery seemed to supply the long-sought explanation. The clinician could now understand (or think he understood) why the nucleus of the facial nerve could suffer so severely and yet the middle and lower face alone be paralysed. The upper face escaped because its nucleus, far away from the main nucleus, was not involved in its degeneration.

There is reason to think, however, that the immunity of the upper facial muscles is not always so complete as from a mere examination of the position and movements of the eyelids it would appear to be. There is not infrequently a degree of paresis of the orbicularis with a greater or less dilatation of the palpebral aperture, and even when this dilatation is not apparent, there may be a considerable degree of paresis as evidenced by the reaction of degeneration in the muscles of the upper face. We have recently, in a case of amyotrophic lateral sclerosis, had occasion to demonstrate a reduction to a nearly equal degree of the electrical reactions in the upper and lower face muscles, in which there did not seem on inspection to be any paresis of the upper face ; and we would draw attention in this connection to the condition of the eyelids and their electrical reaction noted in the remarkable case of diplegia facialis published in this review by Drs Fowler and Rainy (6), and which will be again referred to in another relation. They note that their patient, a child of ten months old, showed nothing particularly wrong with its eyelids before entering hospital ; that during the few days of its stay in hospital before its death the lids could not be voluntarily shut very tightly, but that they covered the eyeballs fully during sleep. In spite of this the electrical responses were at first very feeble and subsequently absent altogether. The entire escape of the upper facial muscles is thus, in our opinion, more often apparent than real. Indeed, we venture to doubt if in any case in which there is advanced degeneration of the muscles of the lower face, the electrical examination of the muscles of the upper face will fail to show some degree of reaction of degeneration. It is also a remarkable fact that degenerations of the oculo-motor nuclei do not appear to be associated with paresis or paralysis of the upper face, as would almost certainly be frequently the case if the nucleus for the latter formed part of the oculo-motor nucleus, as stated by Mendel.

Wilbrand and Saenger (7), in their valuable work on the neurology of the eye, summarise the evidence in favour of Mendel's view. It is not very convincing.

(*a*) Obersteiner (8) found that after destruction of one oculo-motor root the posterior (or inferior) part of the corresponding oculo-motor nucleus was intact. It does not, however, necessarily follow that the part of the nucleus which has escaped is Mendel's centre for the upper face muscles. It is now a well-recognised fact that some of the fibres of the oculo-motor root have a crossed origin from the lower part of the nucleus of the other side. Therefore it is practically certain that division of one oculo-motor root would necessarily leave untouched those cells of the nucleus which are the centre for the fibres crossing from the undivided nerve of the other side. In support of this view we have the discovery by Siemerling and Boedeker (9) that a unilateral paralysis is followed by a degeneration in the anterior part of the nucleus of the same side, and by a bilateral paralysis in the distal—that is, the posterior or inferior part of the nucleus.

(*b*) The clinical evidence quoted by Wilbrand and Saenger (7) is as follows :—Hughlings Jackson described three cases of ophthalmoplegia externa associated with involvement of the orbicularis palpebrarum. Turner observed a case of nuclear ophthalmoplegia, with paresis of the upper facial muscles. Meyer has described an analogous case. Woods mentions a case of ophthalmoplegia externa with ptosis, in which there was a weakness of both orbiculares palpebrarum. Smith found in a case of total ophthalmoplegia a participation of the frontalis and orbicularis palpebrarum. Fuchs, in a case of bilateral ptosis, found also weakness of the frontalis muscles. Hanke noted in a case of bilateral ophthalmoplegia externa, which was either of congenital origin or dated from earliest infancy, that at a later period ptosis and paralysis of the frontalis appeared. Birdsall in a case of external ophthalmoplegia observed a diminution of the electrical excitability of the upper division of the facial nerve.

Such evidence, in the absence of control by post-mortem examination, must be regarded as merely suggestive. It is obvious that by the law of probability such associations of symptoms may occasionally arise without their being dependent on lesions of a single centre. There is nothing in the clinical

history of those cases to exclude multiple lesions either of similar or of different kinds; that is to say, there may have been more than one nuclear lesion or more than one peripheral lesion, or nuclear and peripheral nerve or muscle lesions may have been associated together.

On the other hand, Wilbrand and Saenger quote a case carefully examined by Cassirer and Schiff (10), in which, with *complete degeneration of the oculo-motor nucleus, even in its posterior segment, the function of the upper facial nerve was not in any way interfered with*, and they note, without, however, giving cases, that Bernhardt (11), Sauvineau (12), and Siemerling (13), have stated categorically that they have found the upper facial nerve intact in nuclear ophthalmoplegia. Siemerling and Boedeker (9), after a series of exhaustive microscopical examinations of cases of progressive ophthalmoplegia, have arrived at the conclusion that there is *no evidence whatever that the oculo-motor nucleus has any connection with the muscles supplied by the upper facial.*

Wilbrand and Saenger, while admitting that the question of the upper facial nucleus is not yet settled, are inclined, on the strength of their own observations, to agree with Cassirer and Schiff, Siemerling and Boedeker, etc., in their opinion.

More recently the question has been attacked from another side—namely, from the study of the *reaction à distance* (or axonal reaction), which follows in the cells of origin of a nerve when its continuity is interrupted.[1]

Marinesco (15) in 1898 divided the branch to the frontalis, orbicularis, and corrugator supercilii, and found a degeneration in the inferior part of the facial nucleus, and in the posterior part of its median nucleus. Section of the inferior facial produced degeneration in the external group of the facial nucleus. Complete division of the facial nerve caused axonal reaction in all the cells of the facial nucleus.[2]

[1] This method is calculated to give reliable data when the subsequent examination of the nerve centres is made at a sufficiently short interval after the initial section or destruction of the nerve. It is not of value, however, when an interval of many months, and still less of many years, has elapsed; therefore, such cases as Gianelli's (14), where the frontal branch of the facial nerve was divided fifty years before death, must be set aside.

[2] Marinesco has shown that the common facial nucleus varies in its outlines at different levels of its course, and slightly also according to the animal used. In the

Kotelewski (16), after experimental section of the upper branch of the facial, found degeneration in the dorso-lateral portion of the facial nucleus. This in all probability corresponds to the dorsal portion of the middle group of Marinesco, as the dorsal nucleus seems rather to lie behind the outer than the middle group (see Van Gehuchten's Fig. 487).

Parhon and Nadejdé (17) examined a case of cancer involving the superior facial, and found that what they term the first dorsal group [1] showed cells which were greatly atrophied. (The second and even the third dorsal group also showed less marked changes, which they regarded as due, in some measure at least, to lesions of nervous filaments which do not form part of the superior facial. They describe as belonging to the facial nucleus a group, also described by Wyrubow (18), situated between the common nucleus of this nerve and the nucleus of the sixth pair. They regard this group as the centre of the occipital muscle.)

Parhon and Minea (19) found that the nucleus of the upper facial nerve is represented by the first dorsal group of the nucleus of the seventh pair. They state categorically that the oculo-motor nuclei, including that portion which is situated in the depression of the posterior longitudinal fasciculus, do not send fibres into the trunk of the facial nerve.

All these observers, therefore, are unanimous that the origin of the upper facial is situated within the facial nucleus in the pons, and apparently in its dorsal part.

With regard to the origin of the nerve to the orbicularis oris, it is now universally admitted that the inferior facial nucleus of Lockhart Clarke (2) has no connection with the facial nerve; but the view of Gowers (1) as to the upper part of the hypoglossal nucleus constituting such a centre has met with wide acceptance. He has brought forward strong arguments in its support. He says: "The orbicularis oris and the transverse

middle of its length three cell groups—internal, middle, and external—can be distinguished. The middle group is again sub-divided into an anterior and posterior segment. At the upper and lower extremities of the nucleus the groups become reduced to one. [For illustration, see *Rev. Neurol.*, 1898, p. 30; also Van Gehuchten, *Anat. du Système Nerveux*, 4me ed., pp. 589 to 591; and Quain's "Anatomy," 11th ed., Vol. iii., Part i., p. 152.)

[1] The reference in the *Rev. Neurol.* does not give figures, so that it is not quite clear what the authors mean by the first, second, and third dorsal groups.

muscles of the front of the tongue have a functional relation closer, perhaps, than any other two muscles in the body equally distinct. Neither can be put in action without the other. The orbicularis is always involved in degenerative disease of the hypoglossal nucleus, and escapes in disease of the chief nucleus of the facial, as in a case of acute atrophic paralysis in which the face was involved." . . . "Considerable degeneration has been found in the fibres of the 'loop' of the facial nerve, even when the cells of the chief facial nucleus were but little affected —a mysterious fact. The posterior longitudinal fibres may be normal or partly degenerated (in proportion, it is said, to the affection of the hypoglossal nucleus); especially the longitudinal fibres suffer in the inner part of the reticular formation which are supposed to continue the anterior ground fibres of the cord. The degeneration is always bilateral."

Illustrations of the possible path from the hypothetical centre in the hypoglossal nucleus have been given by one of us (A. B.) (20), by Ferrier (21), and by Purves Stewart (22), who, in his valuable work on the "Diagnosis of Nervous Disease" states that the facial nerve arises from a nucleus in the lower part of the pons, but derives some of its fibres, namely, those for the orbicularis oculi, from the nucleus of the 3rd nerve, and others, namely, those for the orbicularis oris, from the hypoglossal nucleus.[1] On the other hand, however, Oppenheim (23), while figuring the connection from the 12th nucleus to the 8th nerve, regards it as being very improbable.

If it is the case that the nerve to the lower face arises from the hypoglossal nucleus, it must, as Gowers states, undergo degeneration in disease of the hypoglossal nucleus. A complete destruction of one hypoglossal nucleus should cause paralysis of the lips as well as the half of the tongue on the same side. The converse must also follow, that a section or destruction of the nerve of such a character as to produce *reaction à distance* in the main facial nucleus of the pons must also produce *reaction à distance* in that portion of the hypoglossal nucleus in which the lip muscle fibres take their origin. A case, there-

[1] Dr Purves Stewart, in the second edition of his book, which has just appeared, has modified this statement, saying that the cells for the orbicularis oculi extend as high as the nucleus of the third nerve, and those for the orbicularis oris as low as the hypoglossal nucleus.

fore, of complete facial paralysis in which there was total reaction of degeneration in all the muscles of the face would be, if examined sufficiently early, adapted to prove or disprove the alleged association. If it be found that the facial nucleus is alone involved, and the hypoglossal nucleus quite intact, that would be indisputable proof that the hypoglossal nucleus has no connection with the facial nerve. On the other hand, if the facial nucleus were degenerated, but a portion of the hypoglossal nucleus were also similarly affected (the hypoglossal nerve being, of course, in a normal condition), then the presumption would be very strong, or the proof even absolute, that the lip-facial muscles arose from the hypoglossal nucleus.

Similar reasoning would serve to prove or disprove the origin of the upper facial nerve from the oculo-motor nucleus.

The opportunities for clinical observation of such cases and their confirmation by autopsy are exceedingly rare, and therefore the question has remained for a long time in doubt; but we are in a position to describe a case which has been under the charge of one of us (A. B.) during life, and in which the medulla and pons have been examined microscopically by both of us after death, and which appears to us to justify the conclusion that the facial nerve has no origin in either the oculo-motor or the hypoglossal nucleus.

Clinical Case.—A. M., 65, harbour-master; recommended by Dr Dickson of South Queensferry; admitted to the Royal Infirmary, Edinburgh, in February 1908.

Abstract of Notes of History and Examination, as made by Drs Hugh More and W. Kelman Macdonald:—Sudden onset on 1st December 1907, with pains all over his body. Fourteen days later development of giddiness, which gradually increased, producing great uncertainty in walking, with fear of falling and tendency to sway towards the right side, without, however, any actual fall. Simultaneously there developed deafness, with buzzing noises in the left ear, the deafness in a short time becoming absolute. Also left facial paralysis. No headache. No other sensory or motor disturbances.

Previous history was satisfactory, except that his left eye was removed thirty years before for "some inflammatory trouble." Otherwise there was no personal or hereditary taint.

Examination of the face showed a complete left facial paralysis, the left forehead being smooth and immobile, the left

eyebrow lower than the right, and the aperture of the mouth having the characteristic obliquity. The mouth was somewhat drawn to the right side. The palpebral aperture, owing to the absence of the eye, was only slightly open. None of the muscles of the face were capable of any voluntary or reflex movement. There was no response to faradic stimulation. On galvanic stimulation of the muscles a slow and feeble contraction was obtained, but the cathodal closing contractions remained slightly stronger than the anodal closing contractions. The tongue was protruded without lateral deviation. The sense of taste was equally well conserved on both sides of the tongue. The examination of the ear by Dr Logan Turner showed no evidence of any middle-ear catarrh, past or present.

Closer examination of the nervous system showed nothing abnormal, with the exception of some defect of sight in the right eye, associated with an increased depth of the anterior chamber, tremulousness of the iris and hippus. There was no optic neuritis. The movements of the right eye were unimpaired. The stump of the excised eye-ball was capable of a little movement which was communicated to the eye-lids.

The superficial and deep reflexes were normal, the plantar reflex being of the flexor type on both sides.

Further examination revealed evidence of chronic tuberculosis in the upper part of both lungs, some dilatation of the heart, especially in its left ventricle, with a stenosis of the mitral orifice. The heart's action was somewhat irregular, the pulse tension low, the arteries tortuous and their walls thickened and beady. The urine showed a specific gravity of 1014, but it was otherwise normal. There was some evidence of rheumatoid arthritis in the hands, feet, and hip. There was also a reducible left femoral hernia and some tendency to varicose eczema in his lower limbs.

Diagnosis was made of neuritis of the facial and auditory nerve in the internal auditory meatus. The absence of evidence of middle-ear catarrh and the retention of the sense of taste and the association of deafness, giddiness, and facial paralysis indicated that the lesion was probably situated between the side of the pons and the bottom of the internal auditory meatus. The completeness of the facial paralysis was evidence of a total involvement of the facial nerve.

Subsequent Progress.—The patient, who was treated with rest and iodide of potassium, regained strength to a considerable extent, but did not recover from his giddiness, and died suddenly from heart failure on 2nd May 1908.

Autopsy.—The autopsy revealed general fibrous adhesions of pleura, chronic interstitial pneumonia in the upper halves of both lobes, the remainder of the lung showing chronic venous congestion with œdema. The heart was soft and flabby, with fatty infiltration, especially on its right side, dilatation of all its chambers without hypertrophy of the walls except to a slight degree in the left auricle. The mitral valve was dilated, with some thickening of its cusps. The tricuspid valve was also dilated. There was diffuse epicardial thickening, with localised "milk-spots" towards the apex of the left ventricle anteriorly and posteriorly over the lines of the vessels. There was extensive fatty infiltration of the wall of the greatly dilated right ventricle. Both coronary arteries were thickened and showed atheromatous patches. The aortic cusps were dilated, with thickening along their attachments and towards the corpora Arantii. The left ventricular wall was thin, friable, soft and fatty, the papillary muscles being fibrous towards their tips. The liver and kidneys showed a marked degree of chronic venous congestion, the kidneys also showing atrophy of the cortex, increase of pelvic fat, and some chronic interstitial nephritis.

The left optic nerve was atrophied, and the left 3rd, 4th, and 6th cranial nerves were somewhat thinned. There was some slight milky thickening of the arachnoid over the inferior surface of the cerebellum and between the lobes; also to a slight extent over the circle of Willis, and extending 1¼ inches in front of the optic chiasma to near the tips of both temporal lobes.

The brain was carefully hardened in formalin. The pons and medulla were embedded in paraffin, and serial sections were cut of the whole length of the facial, oculo-motor, and hypoglossal nuclei. These were stained throughout with Unna's polychrome blue and by Van Gieson's method.

Within the pons the facial nucleus of the same side was completely affected; not a single cell was found to be normal. The nucleus itself showed a considerable diminution in the number

of its cells. Those that remained had all reached the stage of atrophy, still showing a well-marked axonal reaction, with eccentric nuclei, chromatolysis, and more or less convex outline. Many of them also contained large masses of yellowish pigment granules.[1]

On the other hand, there was no degeneration whatever in the facial nucleus of the opposite side. Its cells were without exception healthy. Many of them contained some yellow pigment, but apart from that, the Nissl's granules and the position and character of the nuclei were quite normal. It is hardly possible to conceive a more positive demonstration that none of the fibres of the facial nerve arise from the nucleus of the opposite side. Had it been otherwise we should have found normal cells in the degenerated nucleus, as well as degenerated cells in the opposite nucleus.

A similar examination of the hypoglossal nuclei demonstrated that their component cells were throughout quite intact. Here and there a cell showed some slight degree of pigmentation, but that was perfectly explainable by the age of the individual. Apart from that, the cells were well formed, their Nissl's granules everywhere presented their normal characters, and the nucleus occupied its normal position.

On examining the oculo-motor nuclei, no distinction could be made out between the two sides. Notwithstanding the removal of the eye-ball, and the resulting comparative inaction of the muscles, *there was no indication of any change in the character of the cells on the affected side.* Their nuclei were invariably central, their form normal, and their Nissl's granules stained with great distinctness. The nuclei on both sides were searched from end to end, and in no part was there any evidence of degeneration. The sections were submitted to several other workers in the Neurological Laboratory, and they likewise failed to find any difference between the two sides.

We might again refer to Drs Fowler and Rainy's (6) case of cerebral diplegia, in which they found, in what was practically a complete bilateral facial paralysis, that there

[1] Almost midway between the main nucleus and the abducens nucleus a small group of motor cells, described by Wyrubow (18), was found to be atrophied on the affected side and normal on the other. They thus evidently belong to the facial nerve.

was no abnormal change in either the oculo-motor or the hypoglossal nucleus, and no degeneration in the posterior longitudinal fasciculus.

Conclusions :—

1. That the upper facial nerve does not arise from the oculo-motor nucleus.

2. That the lip-facial fibres do not arise from the hypoglossal nucleus.

3. That there is no crossed origin of the facial nerve from the main nucleus.

4. That no crossed origin for any of the fibres has yet been discovered.

5. That all the fibres of the facial nerve arise from the groups of cells in the pons which lie behind the superior olive, and are known generally as its main nucleus—these groups being regarded as including the small accessory group situated a little behind it (Wyrubow), *i.e.* nearer to the abducens nucleus.

6. That the upper facial nerve probably arises from the dorsal part of the nucleus.

7. That further localisation of function of the nucleus has not yet been clearly established.

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HÆMATOGENESIS IN FŒTAL SHEEP.[1]

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(Plate XXI.)

I. PRELIMINARY.

The material on which this research was carried out consisted of a series of fœtal sheep obtained from the Edinburgh abattoirs. The larger fœtuses were fixed in 15 per cent. formalin, a few of the smaller in formol-alcohol.

The series comprises fœtuses of the following lengths in centimetres:—0·6, 0·8, 1, 1·5, 2, 2·5, 2·8, 3, 3·5, 4, 5, 5·5, 6, 6·5, 7, 7·5, 9, 9·5, 10, 11, 12, 17, 22, 28, 30, 34·5, 35, 45, 46·5. In addition to these the marrow, liver, and spleen of lambs up to a few days old were examined. The measurements were made from end to end of the small lambs, from the frontal eminences to the root of the tail in the larger, *i.e.* in the longest axis in every case. The specimens were embedded in paraffin. Complete serial sections of the whole fœtus were made in the case of those not exceeding 3 cms. In specimens measuring from 3·5 to 7 cms. serial cross sections were cut in the regions of the thymus and spleen. In the case of all the larger fœtuses portions of liver, spleen, thymus, and bone marrow (or shaft of the femur) were cut separately. Films of these structures were also examined. In the case of the smaller fœtuses the marrow films were made by squeezing the shaft of the femur with forceps and smearing the exudation over a cover-slip.

The stains employed were methylene-blue and eosin, and hæmatein and eosin in the case of the sections. Jenner's and Leishman's stains were also employed in preparing the films.

In the following descriptions, under the different sizes, the specimens are to be taken as representing a group rather than as individuals. The description of intermediate stages has been omitted where the changes were slight or unimportant.

II. DESCRIPTIVE.

Fœtus, 0·6 cm.—At this stage the mesoblastic somites are formed; the primitive heart and kidneys are the only defined organs. Liver being differentiated.

In the heart, among the liver cells, between the kidney tubes and in the connective tissue throughout the body, are large numbers of megaloblasts, red cells showing considerable uniformity as regards size, maximum diameter about 7 μ, with strongly basophil nucleus showing a chromatin reticulum and a number of closely set chromatin masses. The protoplasm contains hæmoglobin,

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evidently in pretty full complement, as only a very slight degree of polychromatophilia is present. Large numbers of these cells are in the various stages of mitosis. No leucocytes of any kind were seen.

Fœtus, 1 cm.—Primitive kidney and liver formed, the other abdominal viscera represented by a few epithelial tubes in undifferentiated mesenchyme. The trachea alone represents the respiratory organs. Thymus not present in most of the specimens, but in one fœtus a slight downgrowth from the fourth visceral cleft may have been an early indication of its formation.

The blood cells present are megaloblasts distributed throughout the connective tissues, specially numerous as would be expected in the auricles, the primitive aorta and its arches, and the umbilical vessels; but in addition they occur in great numbers between the irregular strands of liver cells, between the primitive kidney tubules, and in the perinephritic connective tissue. With one exception no cells of the leucocyte series could be distinguished in any of the sections. The leucocyte seen had a pale round nucleus and basophile protoplasm.

Fœtus, 2 cms.—The thymus has now appeared in the form of short epithelial buds lying in the primitive connective tissue immediately above the pericardium.

The majority of the blood cells in the heart and vessels are megaloblasts similar to those just described, but they show considerable individual differences. The amount of hæmoglobin varies considerably, so that polychromatophilia is marked. In many instances the nucleus appears to be undergoing degeneration and absorption. A small proportion of red corpuscles (megalocytes) without nuclei are now found. In addition to these, two other varieties of blood cells are found in the circulating blood in very small numbers. These will presently be described. Blood cell proliferation (megaloblastic) is still found in the connective tissues, especially in the region immediately anterior to the proto-vertebræ, but is not now nearly so active, and has greatly diminished in the region of the kidney.

The liver has now come strikingly into evidence as a hæmopoietic organ. Among the reticular strands of liver cells are numerous strongly basophile cells without hæmoglobin. These are—(1) Small cells with very darkly stained nuclei, which are characterised by a radial arrangement of thick chromatin strands with chromatin thickenings. The protoplasm consists of a narrow ring of much more faintly stained homogeneous basophil protoplasm. (2) There are also cells with precisely similar characters, but nearly twice as large. The chromatin strands are more widely separated, so that the nucleus appears much paler. The protoplasm is similar to that of the first variety, but is relatively and absolutely greater in amount. Transitions between the two varieties are present, and indicate that the second variety is derived from the first,—that they are older individuals of the same kind of cell. There are indeed areas of these cells where the smaller variety occupies a central position with a peripheral ring of the larger variety, but this arrangement never approaches anything like the definite appearance of a lymphoid germ centre. As regards relative numbers, the larger variety preponderate slightly. Both varieties show mitoses, but these occur in a greater proportion of the small cells. These cells seem to correspond with the *stark basophile schmalleibige kleine Hamatogonie* (erythroblastic) and the *ältere kleine Hämatogonie mit breitem Rand* of Pappenheim (1907). Among the liver cells an occasional cell of the leucocyte type was noticed, but at this stage they are not to be found with certainty even after prolonged search. Those seen resembled the larger erythroblasts as regards size, and had pale vesicular nuclei with a very thin scanty reticulum and two or three very small, round, chromatin points. Their protoplasm was deeply basophilic.

Fœtus, 2·8 cms.—In the vessels a much greater number of cells without nuclei (megalocytes). In liver a great increase in the number of erythroblasts.

Fœtus, 3 cms.—In the auricles the majority of the cells are megaloblasts. These show marked polychromatophilia, and in many the nucleus is disappearing. Megalocytes greatly increased in number. A larger proportion of the basophil erythroblasts present. A few cells of similar type appear to be acquiring hæmoglobin. A few leucocytes, singly, at wide intervals.

Liver.—In the liver very numerous erythroblasts, both large and small. The former preponderate. Mitoses numerous. Here and there throughout the liver are leucocytes, singly or in small groups never exceeding four or five in number. They are considerably larger than the erythroblasts; nuclei rounded, comparatively pale, with a fine chromatin reticulum and a few rounded chromatin masses. Protoplasm relatively more abundant than that of the larger erythroblasts; slightly more basophil and more easily indented and distorted. A few giant cells (myeloplaxes or megakaryocytes) among the liver cells.

Thymus.—The thymus now consists of two portions, one just above the pericardium and another higher up in the neck. These structures are entirely composed of epithelium forming a solid mass with irregular, short projections. The surrounding connective tissue contains numerous blood vessels whose contents are similar to those of the auricle. Among the connective tissue cells a fair number of megalocytes, erythroblasts, and leucocytes, similar to those found in the liver. In one or two instances, however, the leucocytes had an indented or bilobed nucleus.

Fœtus, 4 cms.—*Thymus.*—At 4 cms. the epithelial processes are more branched, and show intersections by young fibrous tissue. This fibrous tissue contains numerous vessels. Among its branching cells and processes are now fairly numerous large primitive leucocytes. These seem to be more numerous than in any other situation except the liver. A few of them are invading the epithelium, and many show mitosis. Cells resembling the smaller erythroblasts are also present in the tissue round the thymus. The question of their identity with these cells will be subsequently discussed.

Fœtus, 5 cms.—The overwhelming majority of cells in the vessels are non-nucleated hæmoglobin-carrying megalocytes. Hæmoglobin-containing megaloblasts also present; many of them show mitotic figures.

Liver.—The common cell between the strands of liver substance is still the erythroblast. There are a few leucocytes, but the number of giant cells has strikingly increased, and transitions between the primitive leucocyte and typical "myeloplax" are common. The latter now attains very large size—a common diameter being 30 μ. The nucleus may appear as five or six separate masses, but connections are always to be made out between these. The nuclear envelope is thick. The chromatin reticulum is scanty. The little rounded chromatin masses are conspicuous. The protoplasm of the smaller giant cells is practically homogeneous. As the cell enlarges a fine reticulum can be distinguished, and as the cell further enlarges the protoplasm becomes more and more granular in appearance and more and more acidophil in reaction. A giant cell is occasionally seen in mitosis. A few of them are phagocytic. One or more megalocytes are occasionally to be seen in a vacuole in the giant cell protoplasm.

Thymus.—A further increase of vascularity; the epithelial masses broken up by connective tissue; a very great increase in the number of cells in the surrounding connective tissue. These cells are the large primitive leucocyte and smaller cells whose characters resemble the small erythroblasts. At this stage it has become possible to differentiate these cells from the erythroblasts, and to recognise them as small lymphocytes. The whole nucleus is paler than

that of the erythroblasts; the chromatin strands are not so thick, and the chromatin masses tend to be larger and more scanty. The protoplasm is rather more basophil. These cells have invaded the epithelium in great numbers, and towards the periphery of the thymus greatly outnumber the epithelial cells. In the connective tissue round the thymus are now little groups of nucleated red cells—some showing mitotic figures.

Sections of femur.—In most specimens ossification has not proceeded further than an enlargement of the cells in the middle of the cartilage. One or two specimens, however, show deposition of a layer of periosteal bone and irruption of the cartilage by vessels and fibroblasts. These vessels contain megalocytes almost exclusively. A very few nucleated cells are present. Among the irrupted osteoblasts are scanty groups of three or four small erythroblasts at wide intervals, and still less frequent is an occasional megaloblast and an occasional primitive leucocyte.

Fœtus, 6 cms.—*Liver.*—Some indication of lobular arrangement; great erythroblast activity; great increase in the number of large primitive leucocytes, groups of four or six being common, while islands of twenty or more occur. Transitions between these cells and megakaryocytes are numerous. The latter sometimes show phagocytosis and rarely mitosis.

Spleen.—The spleen has been differentiated. It consists of a solid mass of branching connective-tissue cells with a few thick-walled vessels. Among the connective-tissue cells are a few primitive leucocytes and erythroblasts and fewer young giant cells. These varieties were always seen singly. A good many megalocytes present in capillaries.

Fœtus, 7 cms.—Liver and spleen resemble those of fœtus 6.

Thymus.—Thymus now divided up into lobules consisting of a central (medullary) portion mainly epithelium and peripheral masses of lymphocytes alone. In the medullary portions a fair number of lymphocytes and a few large primitive leucocytes. A few megaloblasts. The connective tissue around the lobules very vascular.

Sections of femur.—More periosteal bone and a very greatly increased vascularity. The blood cell increase is due to non-nucleated elements.

Fœtus, 10 cms.—*Liver.*—The liver shows definite arrangement of its cells in radiating rows. Portal tracts and bile ducts evident. Between the rows of cells erythroblasts in great numbers. Large leucocytes numerous, and some of them show indentation and lobulation of the nucleus and a much less basophil protoplasm. Indications of granule formation can be distinguished. Giant cells are more numerous than before, and some are phagocytic.

Spleen.—Spleen invested by a thick capsule. Trabeculæ not formed. The bulk of the organ still consists of branching connective-tissue cells, but round the arteries these cells are invaded by blood cells giving rise to small Malpighian bodies. The cells forming the Malpighian corpuscles are mainly primitive large leucocytes, but there are also a fair number of small lymphocytes, as described in the thymus. A very high proportion of both these varieties shows mitosis in this situation.

In the pulp numerous blood cells are scattered throughout, but are never found in large groups. In numerical order the cells found are non-nucleated red elements, erythroblasts, and primitive large leucocytes. Giant cells are fairly common, most of them having only a slightly indented or lobed nucleus and basophil protoplasm, a few having the typical "basket-shaped" nucleus, and more acidophil protoplasm.

Thymus.—No essential difference from the appearance seen at 7 cms. Megaloblasts, erythroblasts, and primitive leucocytes numerous in the connective tissue between the lobules.

Sections of femur.—Cartilage all opened up; thick deposition of young

periosteal bone, and a medullary cavity. In the vessels are numerous megalocytes and very few nucleated cells. Little cellular depositions, most between the invading osteoblasts and young bone. These consist of rather circumscribed groups of erythroblasts numbering twenty or thirty, and more widely scattered, and smaller groups of primitive leucocytes. Some of the latter attain very large size, and some show definite signs of transition to the giant cell type. Some of the erythroblasts are acquiring hæmoglobin. A few of the leucocytes have lobed nuclei and eosinophil granules in their protoplasm. Multinucleated osteoclasts are fairly numerous along the bony trabeculæ. They require no description here, but are not to be confused with the giant cells.

Bone-marrow films.—Prepared by squeezing the shaft of the femur in forceps and smearing the cut end over a coverslip. The majority of the cells were large primitive leucocytes, a fact which indicates that little, if any, of the contents of the vessels had been squeezed out.

Films stained with eosin and methylene-blue showed the same kind of cells as seen in sections, but in films stained by Jenner's method many of the leucocytes contained large basophil granules (basophil myelocytes), and these granules seemed gradually to accumulate in the large primitive leucocyte. The apparent absence of these granules in the sections is probably due to the fact that they are water-soluble.—Gulland (1906). Eosinophil myelocytes were present in smaller numbers.

Fœtus, 12 cms.—*Liver.*—Great increase in the number of non-nucleated red cells. Erythroblasts in great mitotic activity.

Spleen.—Trabeculæ now formed. Large numbers of blood corpuscles throughout pulp.

The next most numerous blood cells are the erythroblasts. These are also scattered throughout the pulp, but here and there occur in groups. Some are tinged with hæmoglobin. A few primitive leucocytes and a very few myelocytes are found. The Malpighian bodies resemble those seen at 10 cms.

Sections of femur.—Cell deposits now larger and more numerous. Most of the erythroblasts acquiring hæmoglobin; a good many megaloblasts with their full complement are present. Eosinophils fairly common; myelocytes and polymorpho-nuclear leucocytes of the neutrophil series in fair numbers. The large primitive leucocytes still numerous, and their numbers are being reinforced by a metamorphosis of the connective-tissue cells of the primitive marrow reticulum. These cells become rounded and project from the fibrous strands. They lose their connection with these strands; their chromatin gradually increases and becomes slightly more basophil, and they are then indistinguishable from the primitive leucocytes.

Films.—The non-nucleated red cells now predominate, and there is a close correspondence to the cells seen in sections.

Fœtus, 17 cms.—*Liver.*—Still enormous numbers of erythroblasts between the rows of liver cells. A very small proportion are acquiring hæmoglobin. Mitoses are numerous. Large primitive leucocytes widely scattered throughout, singly or in small groups; a few of them show indented nuclei and faint granules in the protoplasm. Giant cells very numerous. Some show mitosis.

Spleen.—The spleen in general arrangement has acquired adult characters. Malpighian bodies are conspicuous, and run along the arteries forming a sheath. The pulp is intersected by thick trabeculæ containing numerous visceral muscle fibres. Non-nucleated red cells are numerous in the pulp. Little erythroblastic islands are scattered throughout. A few large primitive leucocytes are to be seen, and some of these are being formed by a differentiation of cells of the reticulum. The leucocytes of the Malpighian bodies now obscure the reticulum, and resemble lymphocytes of the adult type (see Thymus,

5 cms.). Most of them are intermediate in size, between the large and small adult lymphocyte.

Bone marrow. — Sections of the femur now show definite Haversian systems. In the vessels there are large numbers of non-nucleated red cells. Nucleated elements of any kind are uncommon, not so many as one occurring in each $\frac{1}{12}$-inch field.

A great increase in the number of extra-vascular cell elements has occurred. These appear to be about equally divided between the erythroblastic and leucoblastic groups. A large number, probably the majority of the erythroblasts, are now acquiring hæmoglobin. Cells corresponding to the adult normoblast with pyknotic nucleus are fairly frequent. The large primitive leucocyte is in fair numbers, and transitions from it to fully-formed myelocytes and polymorphonuclear cells, both neutrophile and eosinophile, are common. Transitions between it and the giant cell are numerous, but very few of the latter have attained their full development as seen in the liver.

Fœtus, 22 cms.—*Liver.*—Except for a distinct increase in the number of granular leucocytes, the appearances resemble those of fœtus, 17 cms.

Spleen.—Malpighian bodies larger. The erythroblasts are in many cases acquiring hæmoglobin, and the number of granular cells, especially myelocytes, has increased. Basophils here and there in the Malpighian bodies.

Thymus.—There is now a complete ring of lymphoid tissue round each central island of epithelium. This epithelium is greatly invaded by lymphocytes, and the interlobular connective tissue has been greatly curtailed by lymphocyte increase. A few eosinophil myelocytes are scattered throughout, and sometimes occur in small groups.[1]

Bone marrow.—The marrow is much more circumscribed in the medullary cavity. Some fat cells are present. Leucoblastic elements have greatly increased. Primitive leucocytes, myelocytes, polymorphs, and eosinophils numerously represented. Giant cells fairly numerous. Films show in addition a number of basophils. Red corpuscles are found mixed throughout all the other cells and erythroblasts; megaloblasts and normoblasts are present.

Fœtus, 30 cms.—*Liver.*—A slight diminution now occurs in the number of erythroblastic cells, which, however, by far outnumber the other blood cells. Small groups of primitive leucocytes with transitions to polymorphs and eosinophils are common. Giant cells are numerous and very large. A few are phagocytic to red cells and erythroblasts.

Spleen.—Erythroblast elements are scanty. Primitive leucocytes are numerous, and the myelocytes, polymorphs, and eosinophils outnumber them, and outnumber those in corresponding areas of liver. The granules are very scanty in some of the eosinophils.

Thymus.—There is greater lymphocyte invasion of the medulla of the lobules, and a fair number of eosinophil myelocytes are present.

Bone marrow.—Cartilage is now seen only as degenerated strands enclosed in the bony trabeculæ. Fat cells have increased among the blood cells. Leucoblast activity seems to preponderate over erythroblastic. Myelocytes, polymorphs, and eosinophils have greatly increased at the expense of the large primitive leucocytes. Eosinophils occur in fairly large groups. In films these cells are outnumbered by basophils.

Erythroblasts without hæmoglobin have practically disappeared. Megaloblasts and normoblasts, many examples showing mitosis, are numerous. Typical giant cells are fairly numerous.

Fœtus, 35 cms.—*Liver, Spleen, Thymus.*—Hæmatogenetic findings do not differ much from those seen at 30 cms.

[1] A few Hassall's corpuscles are now formed, but these and some similar findings do not fall within the scope of this paper.

Bone marrow.—The marrow has increased in amount, and can be readily scooped out. There is a definite arrangement of wide capillaries containing red corpuscles. Between these are large cellular accumulations forming erythroblastic and leucoblastic areas. These are in no way sharply differentiated, but parts can easily be demonstrated as erythroblastic, myelocytic, or eosinophilic. The myeloblastic areas preponderate. Giant cells more numerous and larger than at any previous stage. A few show mitosis. In the erythroblastic areas are many cells without hæmoglobin, but megaloblasts and normoblasts are also found. In the large myeloblastic areas are many large primitive leucocytes, also cells corresponding to lymphocytes of intermediate size. Myelocytes with very fine pink granulation numerous; polymorphonuclear leucocytes, most with horseshoe nuclei, are abundant.

Eosinophil myelocytes and polymorphs present in smaller areas, and not so densely packed.

In films stained by Jenner's method basophils outnumber the eosinophils.

Fœtus, 45 cms.—*Blood.*—Specimens of heart blood taken some hours after death are necessarily unsatisfactory. The following paragraph describes such a specimen, but although the cells were well preserved their proportions do not necessarily indicate the proportions in the living circulation.

The red cells were of the megalocytic type and slightly polychromasic. Of the nucleated cells, megaloblasts greatly preponderated. About one in every four of these had no hæmoglobin. The next most numerous cells were large primitive leucocytes (one to every three megaloblasts), normoblasts (half as numerous), and polymorphs (still fewer).

Liver.—Little qualitative change in the cells, but hæmopoietic areas have considerably diminished. Many of the giant cells are degenerated.

Spleen.—The Malpighian bodies large. The pulp is packed with blood cells obscuring the reticulum. The large primitive leucocyte preponderates, but granular cells of all sorts are numerous. Megaloblasts plentiful, and a fair sprinkling of normoblasts. Giant cells fewer..

Thymus shows little change.

Bone marrow.—Cell proliferation very active, and all varieties of cells are more intimately associated, so that the areas which can be differentiated as leucoblastic or erythroblastic are smaller. Megaloblasts without hæmoglobin still numerous, but a greater proportion contain it. Giant cells very plentiful and large; many phagocytic. Not more than one cell is usually seen contained in a giant cell. These found ingested were erythroblasts, red corpuscles, and polymorphs.

Fœtus, 46·5 cms.—This specimen had a well-developed fleece, and appeared to be approaching full time.

Liver.—Hæmopoietic areas numerous, but more limited to the peripheral parts of the lobules, and very much smaller in size. Erythroblast areas are the more numerous. These comprise erythroblasts of which a fairly large proportion contain hæmoglobin, and a large number of fully developed red cells (mostly megalocytes). Mitoses very numerous. The leucoblastic areas are commonly composed of groups of about four primitive leucocytes, and often in addition a myelocyte, polymorph, or eosinophil. Each of these varieties also occurs singly throughout the liver. The number of giant cells has notably diminished.

Spleen.—The Malpighian bodies are large. The pulp is densely packed with cells. Most numerous are large primitive leucocytes, then cells of the granular series taken collectively, erythroblasts, myelocytes, and polymorphs, and lastly eosinophils and basophils in about equal proportions.

Thymus.—Lobules large; the proportion of cortex in each lobule has increased. Large primitive leucocytes and small lymphocytes with denser chromatin present in about equal numbers. In the medulla fair numbers

of eosinophils, usually with small rounded nuclei. Basophils also present in slightly smaller numbers; an occasional polymorph.

Bone marrow.—Cells in the marrow densely packed, and show a picture of remarkable reproductive activity. Fully formed red corpuscles are most numerous. Next most common are erythroblasts varying greatly in size, and fewer megaloblasts. A few normoblasts containing hæmoglobin present. The large primitive leucocyte is numerous, and shows transitions as before to myelocytes, polymorphs, and eosinophils. The increase of the latter is perhaps the most striking feature at this stage. Basophils are considerably fewer. A good many cells show notched or horseshoe nuclei with feebly basophil or hyaline protoplasm — "transitional cells." Giant cells are now very numerous and very large. They do not appear to be so actively phagocytic as at earlier stages.

Lamb, 1 day old.—*Liver and Spleen.*—These organs do not now contain hæmopoietic areas, and resemble those of the adult.

Lamb, 3 days old.—*Marrow.*—Erythroblasts without hæmoglobin still fairly common. Normoblasts are slightly more numerous, and eosinophils are rather more numerous, but the general picture closely resembles that seen in fœtus, 46·5 cms.

Lamb, 22 days old.—*Bone marrow.*—A very striking increase of fat has occurred, so that the hæmopoietic cells are divided up into little irregular areas between the fat cells. These areas are composed of erythroblasts, many of whose nuclei now approach the pyknotic normoblast type, undifferentiated leucocytes, and their granular descendants. Ordinary polymorphs are strikingly increased, and occur in fairly large groups. Their granulation is exceedingly fine. There are also very large groups of eosinophils, which far outnumber the neutrophil polymorphs. Basophils occur in much smaller numbers.

Hæmatogenesis in a Human Fœtus.—I have recently had the opportunity of examining serial sections of a human fœtus. Unfortunately it had not been measured, and the data regarding its age were unsatisfactory. The spleen had just been differentiated, the cartilages were vascularised, but no marrow deposition had occurred. The liver showed erythroblastic areas, smaller leucoblastic areas, and numerous young giant cells. The thymus showed active lymphocyte proliferation.

In these organs the cytological conditions were exactly comparably to those found in a fœtal sheep measuring about 5 cm. It is therefore justifiable to refer to points in human embryology and pathology which have a bearing on the following discussion.

III. THE SEQUENCE OF DEVELOPMENT AND RÔLE OF THE HÆMOPOIETIC ORGANS.

A. Sequence of Development.

Up to 2 cms. proliferation of megaloblasts is taking place in the vessels and mucoid tissue throughout the body, so that no particular organ can be said to have a specially hæmopoietic function. The proliferation that is taking place in the liver and kidney is in no way different from that occurring in the vessels.

From 2 cms. onwards throughout the greater part, if not the whole, of fœtal life the liver is the chief seat of hæmatogenesis. Erythroblastic function is then evident. Leucocytes are present, but

do not occur in proliferating groups till the fœtus attains a length of 6 cms.

At 4 cms. leucocyte formation begins in the region of the thymus. At this period leucocytes are probably more numerous in the connective tissue round the thymus than in any other part of the body, as Gulland (1891) has shown, although the liver might be a possible exception.

I am in agreement with Bryce (1905) and Hammar (1905), and in opposition to Beard (1900, 1902), in finding that leucocytes are present in the body at a period when the thymus is still a purely epithelial organ. It should be noted that the first appearance of leucocytes in the region of the thymus occurs in the connective tissue surrounding it, and not among the epithelial cells. This appearance suggests a chemiotactic process. My reading of the appearances described is as follows: The epithelium of the thymus becomes surrounded and afterwards invaded by large primitive leucocytes and the products of their division, the lymphocytes, which thereafter actively divide and supply lymphocytes *via* the lymphatic vessels to the circulation. I am unable to agree with Stöhr (1905) and Basch (1906) that the so-called lymphocytes are epithelial in nature and origin. The occasional finding of little groups of eosinophils and basophils indicates that the primitive leucocyte reaching the thymus may occasionally develop along other lines. Groups of eosinophils have been described in the human thymus by Schaffer (1891), Dudgeon (1905), Browning (1905), and others. Not only are white cells other than lymphocytes to be found in the connective tissue round the thymus, but little groups of erythroblasts were noticed in one specimen. This has also been noticed by Schaffer (1893). The production of erythrocytes or of leucocytes of the granular series, however, is not an important or constant function of the thymus, and in the case of the former is probably due to the chance invasion of one or more erythroblasts at a time when these cells in a state of active proliferation frequently find their way into the circulation.

At stage 5 cms. the first indication of hæmatogenesis appears in the femur. The blood cells contained in the vessels which first channelise the cartilage are hæmoglobin—carrying and practically entirely non-nucleated.

At stage 6 cms. the spleen is differentiated, but only contains an occasional erythroblast and leucoblast. It is, however, now a hæmopoietic organ in the same slight degree that the bone marrow is at 5 cms. The bone-marrow therefore anticipates the spleen by a short interval as a producer of erythrocytes and leucocytes. It is not till a size of 10 cms. is reached that hæmatogenesis in either situation can be said to be active and Malpighian bodies are formed.

Lymphatic glands.—These were unfortunately not preserved. It may be stated, however, that no structure resembling a lymphatic

gland was present in any of the serial sections up to 3 cms.; nor were any found in the incomplete serial sections made up to 7 cms.

B. The Association of the Hæmopoietic Organs with the different Blood Cells.

Liver.—The liver is identified with the production of all varieties of blood cells, but its erythroblastic function preponderates throughout. When differentiation of leucocytes occurs, all varieties, with the possible exception of the small (or differentiated large) lymphocyte, occur as soon in the liver as elsewhere. The groups of granular cells, eosinophils, and later polymorphs (possibly also basophils) are never quite so large as those seen in the marrow, especially in the later stages. In consideration of the relatively large size of the liver, however, its importance as a leucoblastic organ as well as an erythroblastic organ must be very great. The hæmogenetic function of the liver terminates somewhat abruptly before birth.

Marrow.—As in the liver, erythroblastic formation preponderates in the marrow throughout the greater part of fœtal life. Towards full time the proportion of leucocytes of the granular series gradually increases, but it never equals that of erythroblastic cells. The activity of the marrow towards birth, and for at least three days after birth, appears very much greater than in the adult condition, as the amount of fat is negligible. It is, however, hardly probable that the subsequent separation of the blood-forming cells into islands by fat cells really indicates any diminution of hæmogenetic activity. The bulk of the marrow increases vastly with increase in the diameter of the bones, and this necessitates the development of an indifferent tissue—the fat, and at the same time allows of elasticity in the arrangement for an increase in blood-forming elements should the necessity for it ever arise.

Spleen.—As a hæmogenetic organ in fœtal life the spleen is comparatively unimportant. The lymphocyte production in the Malpighian bodies does not begin till long after their production in the thymus. Erythroblastic formation at first preponderates in the pulp, but later is exceeded by proliferation of leucocytes.

Lymphoid tissues.—It is not a little remarkable that the lymphocytes, which of all the leucocyte group preserve most closely their resemblance to the primitive type, are the most exclusive as regards the sites of their development. In the sites studied (the thymus and Malpighian bodies of the spleen) the occurrence of any other variety of cell was of great rarity. These cells are also comparatively uncommon in the sites of erythrocyte and granular leucocyte formation.

At the same time, this exclusiveness is not absolute. Cells of the granular series may occur in lymphoid tissue, as has been discussed in connection with the thymus, and lymphocytes were noted in the bone

marrow at stage 35 cms., and transitional cells which belong to this series were found at a later stage. It is also possible that some of the cells without granules and with notched nuclei seen in the liver were "transitional" cells of the lymphocyte series.

Genealogy of the Red Corpuscles.

As my material does not extend back to the period when red cells are first formed, all that can be said is that from 0·6 cm. to 2 cms. megaloblasts and almost no other cells are found in the heart and vessels. Great numbers show mitosis, and are therefore actively proliferating there. At 2 cms. this means becomes insufficient for the necessary supply of blood corpuscles. A new set of cells, erythroblasts, arise which are devoted to the process of supplying them. These cells do not contain hæmoglobin, for the probable reasons that it is a hindrance to mitosis; and, secondly, because the cells being stationary have no respiratory function and do not require it. These cells are first found in the liver, and the question arises whether they are formed there, possibly by a metamorphosis from the reticulum, or whether they are descended from the proliferating hæmoglobin containing megaloblasts.

Bryce (1905) discusses this question in connection with the spleen in his researches on *lepidosiren*, and concludes for the former alternative, for the reasons that the number of erythroblasts is out of proportion to the number of megaloblasts showing mitosis, and for the more cogent reason that the nuclei of the erythroblast differs from that of the primitive megaloblast.

The question is difficult, but I conclude for the alternative that the erythroblasts are the direct progeny of the first megaloblasts in fœtal sheep. I can distinguish no difference in nuclear structure, and for the reasons just indicated there can be little doubt that the erythroblasts can and do proliferate much more rapidly than the first megaloblasts. The erythroblasts having arisen may proliferate for many generations without hæmoglobin, or may begin to acquire it almost at once. Mitosis may occur in these hæmoglobin-containing cells (second megaloblasts), which are indistinguishable from the first megaloblasts found before erythroblasts are developed. This, however, is uncommon as compared with erythroblast division.

A more common appearance between the stages of 2 and 5 cms. is that of megaloblasts with their nuclei undergoing degeneration and absorption, so that by the time a size of 5 cms. is reached the great majority of blood corpuscles in the circulatory system are non-nucleated.

A notable feature throughout the series after stage 5 cms. is the scantiness of nucleated elements in the heart and larger vessels, and the apparent reluctance with which nucleated elements leave the hæmogenetic areas. One is tempted to apply the dictum of Heinz

(1901), that in pathological conditions the appearance of nucleated red cells in the circulating blood indicates a disturbance rather than great activity of the blood-forming elements. The proportion of circulating nucleated cells in no way represents the activity of proliferation, and their presence is in all probability not so much due to the proliferation as to the disturbance it causes.

This thesis has been recently developed by Bunting (1906), who shows experimentally that normoblasts appear in the circulation after marrow disturbance, and that a severe toxic action leads to the appearance of megaloblasts.

Normoblasts.—It is difficult to say exactly at what period normoblasts (hæmoglobin-containing cells with pyknotic nuclei) appear. Normoblasts are certainly present at 35 cms., but long before that, as early as stage 17 cms., certain of the nucleated red cells have a nucleus of normoblastic type. In many instances, however, one could identify them as megaloblasts on account of their large size.[1] The normoblasts are derived from the megaloblasts by their mitotic division. The process is by no means always an abrupt one, and consequently intermediate forms are commonly met with. Normoblasts always contain hæmoglobin to such an amount as to obscure the structure of their protoplasm.[2] They therefore do not show polychromatophilia of the same nature as the megaloblasts, but do show a polychromatophilia in disease due to degeneration.

Megaloblastic blood formation persists, not only to the end of, but for some time after, fœtal life. It is indeed open to question whether it ever disappears entirely. It does not at all follow that megalocytes are supplied to the circulation. The megaloblasts give rise to normoblasts; the normoblasts, in their turn, giving rise to erythrocytes.

The exact mechanism of this latter change I have not followed. In some way the nucleus disappears, and in old cells shows varying forms of degeneration. Extrusion of the nucleus probably never naturally occurs. Extruded nuclei are met with very rarely, and almost always in circumstances indicating that the process has been artificial.

Genealogy of the Leucocytes.

An occasional leucocyte was found in the heart at 1 cm. Fair numbers were found in the liver at 2 cms. Their numbers afterwards increased rapidly. There can be little doubt that their numbers increased out of all proportion to the number of mitotic figures they showed. It is therefore necessary to look for a source from which

[1] Pappenheim has shown that mere size is not a sufficient criterion to distinguish between megaloblasts and normoblasts. I take it, however, that a cell whose dimensions are well above the erythrocyte size may, in cases of doubt, be classed as a megaloblast. The fact that a cell is of ordinary size, however, in no way justifies the opinion that it is not a megaloblast if its nucleus is of megaloblast type.

[2] "Vital" staining is not taken into consideration.

they could be differentiated. Only two suggest themselves. These are—(1) the erythroblasts, and (2) the cells of the reticulum (mesenchyme cells).

I exclude the first source for two reasons. Firstly, no evidence of transition in the form of nucleus, either as regards arrangement of chromatin or tinctorially, although carefully looked for, could be made out. Secondly, the groups of leucocytes were seldom found in relationship to the groups of erythroblasts, but were scattered singly or in little islands by themselves. The alternative remains that the leucocytes were differentiated from mesenchyme cells in or among the liver reticulum. This process I could never actually demonstrate with certainty, but the grouping at an early stage and the arrangement of these cells in relationship to the liver cells suggested a local origin rather than an invasion from without or the result of proliferation. It has been noted, however, that a process of differentiation of leucocytes from connective tissue cells could be followed at a later stage in the developing bone marrow.

From the point of the development of the primitive large leucocyte I am in agreement with most recent authors in deriving from it all the other varieties.

An early cell to appear is the small lymphocyte, which arises by mitotic division of the primitive leucocyte, first in the neighbourhood of the thymus. The small lymphocytes rapidly proliferate, and form the masses of lymphoid tissue of the thymus, spleen, and other organs, and in these organs all the other cells of the lymphocyte group are produced.

The question of the development of the large from the small lymphocyte has recently been much discussed. Pappenheim (1905) distinguishes between these cells by the type of nucleus, and states that although small individual large lymphocytes, and large individual small lymphocytes occur, this nuclear difference is still sufficient to classify them, and he does not admit that the large lymphocytes can be derived from the small.

So far as I can gather from my present material I am inclined to agree with Türk (1905) and Gulland (1906), that the large lymphocyte is developed by growth or in part by absorption of fluid (Gulland) from the small lymphocyte. Among my reasons for this is the fact that in the early development of the thymus the large primitive leucocytes, after dividing to form the small lymphocytes, almost or altogether entirely disappear, and the only cells found there are proliferating small lymphocytes. At a stage somewhat later all the cells of the lymphocyte group are present in considerable numbers. These must be derived from the small lymphocytes by their growth.

I agree with both Gulland and Pappenheim in holding that the large lymphocyte may become either a large mononuclear (splenocyte, in the new nomenclature), or a transitional cell with

notched nucleus. The mechanism of this change is admirably explained by Gulland (1906): "In the large mononuclear the protoplasm increases in amount more rapidly than the nucleus does; the strands of cytoplasm are much more widely separated, and from the large size of the cell the astrosphere is able to lie in its centre without deforming the nucleus to any great extent. In the other case the nucleus increases in size more rapidly than the cell body, and in accordance with Heidenhain's law becomes first indented, then horseshoe-shaped, and ultimately assumes the more markedly polymorphous forms which are found in the transitionals in the blood."

At stage 10 cms. eosinophil granules begin to accumulate in the primitive leucocytes as seen in section, but in films stained with alcoholic mixtures basophils are more numerous. The basophils, therefore, probably appear rather before the eosinophils. As development proceeds eosinophils become much the more numerous. These cells are now myelocytes. Before granules begin to accumulate the primitive leucocyte shows a less basophil protoplasm, so that in many instances it is possible to distinguish a cell as a pro-myelocyte, or in the terminology of Pappenheim's recent comprehensive schema (1907), a myeloblast. This distinction is easier at a later stage in connection with the neutrophil series after all the other cells have been differentiated. As is well established, the myelocytes undergo change in the shape of their nuclei, and become polymorphonuclear cells.

The cells of the neutrophil group are the last to appear. They are seen at 12 cms. in the liver and marrow. Granules, which in the sheep are excessively fine, appear in the protoplasm of the primitive leucocytes, giving rise to neutrophil myelocytes. The intermediate stage of myeloblasts is frequently apparent. From the neutrophil myelocyte the fully developed polymorphonuclear neutrophil leucocyte arises.

Fate of the Primitive Leucocyte.

Towards the end of fœtal life this cell is still abundant in the liver and spleen, and at a later period it is present in the bone marrow. Is this cell to be identified with the large lymphocyte of the adult circulation? I am inclined to answer, no.

As has been seen, there is a period in the history of the lymphoid masses of the thymus and in the Malpighian bodies of the spleen when large lymphocytes are practically absent. They return when the proliferation of small lymphocytes in these situations has slackened a little and permitted individual growth. The great difficulty, however, lies in the practical identity of appearance between large lymphocytes and primitive leucocytes.

As regards this point, I admit that in most instances it is

impossible to distinguish them; yet between typical examples there is a difference, particularly in the greater amount of chromatin in the lymphocyte nucleus. I agree with Türk (1905), that with ordinary stains it is not usually possible to say whether any one individual cell is a primitive leucocyte or a large lymphocyte, but a study of the whole preparation, as a rule, clears up the point.

The fact that eosinophil islands occur in the thymus has already been attributed to the accidental invasion by a primitive leucocyte with a wrong or at least a non-lymphocyte-forming propensity. Were this cell a common element in the circulating blood, such accidental contingencies might be more common (and do occur in leucocythæmia).

I hold, therefore, that the circulating large lymphocyte has been developed from one or other of the individuals of the differentiated lymphocyte group.

The primitive leucocyte remains a normal constituent of the bone marrow, and retains its potentialities of producing all the members of the white cell group. This view brings the history of this primitive cell into close analogy with that of the megaloblast. The primitive megaloblast is at an early stage the circulating blood cell. It soon, however, gives rise to the erythroblasts, and later their normoblast progeny which supply the circulation, while the megaloblast line is continued as a non-circulating element. Megaloblasts escape into the circulation in fœtal life with some frequency owing to changes in the marrow reticulum caused by cell proliferation, but this is to be regarded as an accidental occurrence. Any "accident" (toxin, etc.) in adult life causing a proliferation of the megaloblast rather than its normoblast descendant gives rise to pernicious anæmia.

The Relationship of Leucocytes to Red Cells.

The question as to the possible origin of red cells from leucocytes has recently been fully dealt with from the one side, in a paper by Gulland (1906), which has been commented upon from the other side by Pappenheim. A satisfactory study of the problem would, moreover, demand a much wider purview, phylogenetic and pathological, than that of our present material. I therefore limit myself to the indications which seem to be afforded by the present embryological data. Two points stand out clearly—

1. At stage 2 cms., when megaloblasts are actively proliferating in the circulation, leucocytes are so few as to be practically non-existent, and the occasional leucocytes seen later show no evidence of division.

2. At stage 3 cms., when leucocyte proliferation in the liver has begun, the islands of erythroblasts and leucoblasts are definitely separate in position, and the distinctness of their identity is obvious, and no transitions between them can be seen. These facts argue

strongly against the view that the erythroblasts are derived from the primitive leucocyte.

As regards the first point, I admit that the primitive leucocyte can give rise to a cell which may be locally or temporarily more prolific than itself, *e.g.* the small lymphocyte as seen in the thymus and spleen, but the primitive leucocytes are in enormously greater proportion to these than they ever are to the erythroblasts, and by the time small lymphocytes are proliferating the primitive leucocytes are in active mitosis.

As regards the second consideration, the question becomes more difficult as development proceeds, because the cells become more densely packed, and erythroblast and leucoblast areas encroach on each other. There is no doubt that cells such as Pappenheim has described as large lymphocytes with megaloblast nucleus, etc., and which he regards as transitional forms, exist, but I am not prepared to admit that they are transitional. I have seldom had difficulty in assigning them, in respect of their position or appearance, to either the leucocyte or erythroblast group, and in accounting for departures from type by phases of mitosis or the age of particular individual cells.

We may take it the red cells and white cells are derived from a mesenchyme cell. Their descent, however, proceeds along different lines, and the erythroblasts have been proliferating through many generations before leucocyte proliferation begins. The leucocytes are present in far too small numbers during the preceding stages to give rise to the large numbers which subsequently appear in the liver, and which are differentiated from mesenchyme cells of a younger generation than those which gave rise to the first erythroblasts. We cannot therefore trace back red cells and leucocytes to a common (large lymphoid) parent. Their closest relationship is not brotherhood, but cousinship.

Development of the Giant Cells.

Giant cells first appear in the liver, and are found there at stage 3 cms. They are found in the spleen at 6 cms., and in the bone marrow at 10 cms. They are therefore developed in these various organs just after leucoblastic activity has been established, and there is no difficulty in tracing their origin from the primitive leucocytes. This view is in accordance with that of Muir and Drummond (1893), Heinz (1901), and more recently Hutchison and Miller (1906), who could trace the transition in a case of polycythæmia.

The protoplasm at first is small in amount, but rapidly increases, and as growth occurs the basophil protoplasm turns paler and eventually becomes acidophil. The nucleus becomes indented, lobed, and convoluted, and finally assumes the form of a connected series of circularly arranged masses of chromatin, often forming a horseshoe shape as seen on section. These cells are never multinucleated, as

Hill (1905) has recently stated. They divide by mitosis. The mitotic figures are usually but not exclusively multipolar. I am not convinced that amitotic division occurs, although it has been figured by many observers. I have never seen any appearance that would in any way suggest that polymorphonuclear leucocytes and other varieties are derived from a portion or portions of the giant cell containing one or more nuclei, as has been recently concluded by Walker (1906).

Any form of cell division is a comparatively rare phenomenon among the giant cells. Degenerative changes are common. It is therefore probable that the numbers are recruited only to a small extent by mitosis of giant cells, and to a much larger extent by the growth of primitive leucocytes.

Function of the Giant Cells.

This is largely a matter of speculation, but the associations of these cells may indicate the directions in which their function is to be sought.

Two facts regarding them emerge from this research. (1) They are derived from the primitive leucocyte. (2) They are actively phagocytic.

Their phagocytic action is first seen in the liver (at stage 5 cms.), where they are first developed, and later in the spleen and marrow. In the late stages of intrauterine life their numbers diminish in the liver and spleen and increase in the marrow. The increase noted in the marrow at stage 46·5 is not associated with a corresponding increase in phagocytosis. The majority of the cells taken up belong to the erythrocyte series, but leucocytes are also seen. Lengeman (1901) describes the process of phagocytosis on the part of giant cells by the throwing out of pseudopodia which encircle the smaller cell. I have seen numerous examples which would accord with this view.

Before referring to some of the theories regarding them, some of the other associations of the giant cells may be mentioned. They are to be found in the normal red marrow of at least all the common mammals. They are absent in the marrow of birds. They are present in the adult spleen in a large variety of animals. They have been found in the thymus (Watney, 1882). They occur in the liver and spleen in some cases of pernicious anæmia, Gulland and Goodall (1905),—and in the course of a research on myelocythæmia now in progress they have been met with in lymph glands, and in one case in the kidney. In these cases they were found in association with centres of hæmogenetic activity.

Among other views the giant cells have been thought to produce erythroblasts, to produce leucocytes, to absorb the nuclei of erythroblasts, to be merely the products of certain conditions of nutrition. Their close association with erythroblastic areas has also suggested that they are in some way concerned with the elaboration

of hæmoglobin, and Ciacci and Pizzini (1905) suppose that the splenic giant cells have an influence on the secretion of trypsinogen. None of these views can be said to have much support in fact.

On the other hand, their phagocytic property is obvious during fœtal life, but this function is not so active in the later stages, and is comparatively inconspicuous in adult life when the cells are numerous in the marrow.

In view of their origin from undifferentiated leucocytes the comparative rarity of cell division and the comparative frequency of degenerated forms, I consider that the giant cells are merely the oldest individual form of primitive leucocytes, which have never become differentiated, which have failed to divide at an early stage, and only divide at a late stage with comparative infrequency. Their functional rôle in adult life is unimportant, but in fœtal life they play a part in taking up damaged or effete cells. This function is at first a fairly active one, but it diminishes with the increasing development and activity of the spleen and hæmolymph glands, but even in adult life it is probably not altogether dropped.

SUMMARY.

1. In fœtal sheep measuring from 0·6 to 1 cm. hæmatogenesis consists in a proliferation of hæmoglobin-containing megaloblasts in the vessels and connective tissues.

2. The sequence of development of hæmatogenetic function in the various situations may be tabulated as follows:—

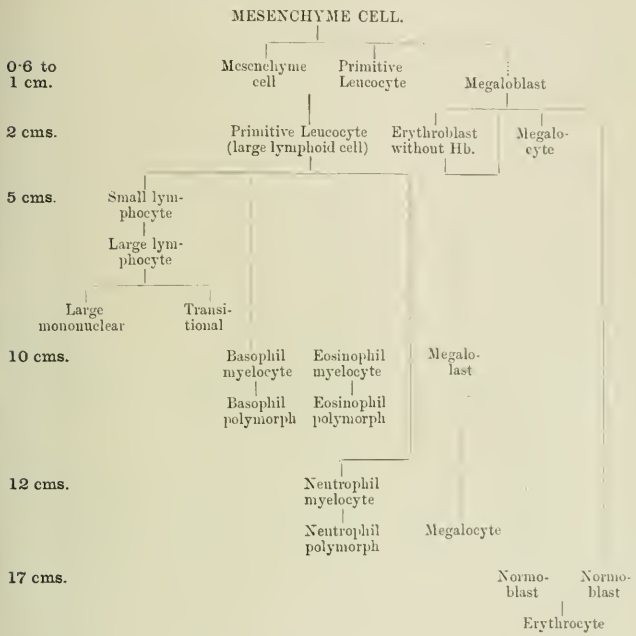
2 cms.	Erythroblastic formation in the liver.
2 cms. to 3 cms.	Occasional leucocytes in the liver.
3 cms.	Leucoblastic formation in the liver.
3 cms.	Giant-cell formation in the liver.
4 cms. to 5 cms.	Fairly numerous leucocytes round the thymus.
5 cms.	Active lymphocyte production in the thymus.
5 cms. to 10 cms.	Slight erythrogenesis and leucogenesis in marrow.
6 cms. to 10 cms.	Similar changes and formation of Malpighian bodies in the spleen.
10 cms.	Active hæmatogenesis with giant cell formation in spleen and marrow.

3. The liver is the most important hæmogenetic organ (erythroblastic and leucoblastic) in fœtal life, and no diminution in the activity of its hæmogenetic function occurs till near the end of intrauterine life.

4. The thymus lymphocytes are derived from the primitive leucocytes, and not from hypoblastic epithelium. Leucocytes are numerous in the liver and elsewhere before they appear round the thymus epithelium.

5. The genealogy of the red and white cells and their relationships to each other are as follows:—

(I have not studied the transition indicated by the dotted line.)



The parent forms persist after the appearance of their more differentiated descendants.

6. The giant cells are derived from the undifferentiated primitive leucocytes, and represent their final stage when they fail to divide or differentiate.

7. They are actively phagocytic towards damaged or effete blood cells in early fœtal life. This function afterwards diminishes, probably owing to the increasing development of the spleen and hæmolymph glands.

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DESCRIPTION OF PLATE XXI.

Fig. 1.—Primitive megaloblasts from heart of fœtus, 1 cm.

Fig. 2.—Erythroblasts.

Fig. 3.—Two polychomatophil (secondary) megaloblasts and a normoblast.

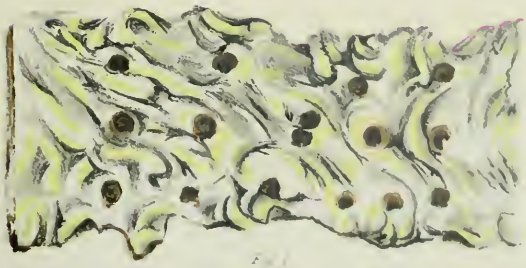
Fig. 4.—(1), A primitive leucocyte; (2) (3) (4) (5) (6) stages in its transition to become a giant cell; (7), a pro-myelocyte; (8), a neutrophil myelocyte; (9), a polymorph; (10), an eosinophil myelocyte; (11), an eosinophil polymorph.

Fig. 5.—Two small lymphocytes, and to the left a large lymphocyte.

(All as seen in section stained with eosin and methylene-blue.)

TO ILLUSTRATE DR. GOODALL'S PAPER.

TO ILLUSTRATE PAPER BY DRS. HEWLETT AND DE KORTÉ.



THE NUMBERS, PROPORTIONS, AND CHARACTERS OF THE RED AND WHITE BLOOD CORPUSCLES IN CERTAIN ANIMALS.[1]

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A great many observations on the blood of the various domestic and commonly used laboratory animals are to be found scattered throughout the magazines, but no very exhaustive account has yet been published. The following data are derived from my own observations and from various sources.

Blood counts in laboratory animals are apt to vary considerably from different causes. The differences are sometimes due to individual peculiarity or to differences in age, feeding, or environment, and it should be remembered that anæmia and parasitic conditions are common and may modify the blood.

Table I.

Animal.	Observer.	Red Corpuscles per c.mm.	Hæmoglobin as percentage of Human.	Leucocytes per c.mm.	Polymorpho-nuclear Leucocytes.	Lymphocytes.	Eosinophils.	Basophils.
Horse . .	Burnett.	7,500,000	90	10,000	60	35	4	1
,, . .	Sabrazès.	8,068,000	73	6,433	37·5	58	2·5	2·5
,, . .	Medizincesci.	...	...	10,000	32–60	34–69	2–6	0·2
Ox . .	Burnett.	6,500,000	60	8,000	30	55	14	1
Sheep . .	,,	10,000,000	...	7,500	38	60	1·5	0·2
Goat . .	,,	14,000,000	...	12,000	...	...	...	...
Pig . .	,,	7,000,000	...	13,000	39	55	5	1
,, . .	Giltner.	8,450,000	88	19,000	37	56	5·2	1·3
Dog . .	Author.	5,599,000	90	19,500	63	30	7	...
,, . .	Burnett.	6,500,000	...	10,000	63	25	12	..

[1] Received June 21, 1909.

TABLE I.—*Continued.*

Animal.	Observer.	Red Corpuscles per c.mm.	Hæmoglobin as percentage of Human.	Leucocytes per c.mm.	Polymorpho-nuclear Leucocytes.	Lymphocytes.	Eosinophils.	Basophils.
Cat . .	Author.	8,000,000	70	18,000	54	37	9	...
,, . .	Burnett.	8,000,000	...	13,000	55	40	5	...
,, . .	Busch and v. Bergen.	6,609,000	...	13,330	60	39	0·9	...
Rabbit . .	Author.	5,160,000	74	10,500	43	52	2·5	2·5
,, . .	Burnett.	5,500,000	74	8,500	47	48	3	2
Guinea-pig .	Author.	5,600,000	100	9,170	37	60	3	...
,, .	Burnett.	5,500,000	...	9,000	47	50	2	1
Rat . .	Author.	8,100,000	110	10,600	28	68	3	1
Mouse . .	,,	10,850,000	90	5,000	23	71	5·75	0·25
Llama . .	Biffi.	11,546,600	...	10,741	75	22·8	1·5	...
Hedgehog .	Author.	8,800,000	...	8,500	37	56	6	1
Fowl . .	,,	3,200,000	...	19,000	37	56	6	1
,, . .	Burnett.	3,000,000	...	25,000	27	65	5	3
Frog . .	Author.	500,000	...	11,000	7	59	27	7
Skate . .	Fraser Harris.	350,000	...	27,500	...	...	...	...

In the case of the more commonly used laboratory animals it may be of interest to state the extremes which I have found in healthy conditions.

TABLE II.

Animal.	Red Corpuscles.	Hæmoglobin.	White Cells.	Polymorphs.	Lymphocytes.	Eosinophils.	Basophils.
Dog	5,500,000	110	31,200	60	25	14	...
,, ...	4,750,000	75	11,200	78	20	2	..
Cat	8,600,000	80	38,800	74	20	6	...
,, ...	7,280,000	65	15,800	40	56	4	...
Rabbit	5,760,000	90	16,000	50	48	1	1
,, ...	4,560,000	70	7,200	32	62	4	2
Guinea-pig . . .	6,880,000	120	...	21	71	8	...
,, . . .	4,800,000	90	...	52	42	6	...

The number of white cells in guinea pigs seems to increase with the age of the animal, as the following table indicates :—

TABLE III.

I.		II.		III.		IV.	
Weight in grms.	Leucocytes per c.mm.	Weight.	Leucocytes.	Weight.	Leucocytes.	Weight.	Leucocytes.
110	7,400	230	8,400	320	11,200	450	8,800
140	11,640	230	8000	325	8,200	460	15,400
150	3,000	260	6,400	335	6,600	480	5,800
155	12,400	285	6,600	390	14,000	530	10,400
160	7,800	290	9,000	380	10,200	570	14,800
170	2,400	...	...	395	5,600	670	9,600
170	2,600	...	...	...	...	675	16,400
175	8,800	...	...	...	...	680	20,400
185	2,800	...	...	...	...	790	17,000
Average.	6,537	...	7,680	...	9,300	...	13,177

Horse and Ass.—Red corpuscles measure 5·5μ. The polymorph nuclei show an unusual degree of lobulation. The neutrophil granules are very fine. The eosinophil granules are spherical or ovoid and are very large.

Ox.—Red corpuscles measure 5·6. General morphology does not differ greatly from that of human blood.

Sheep and Goat.—Red corpuscles measure 5 and 4·1μ respectively. The neutrophil granules are closer together and are very much smaller than in human polymorphs.

Pig.—Red corpuscles 5·6μ. (8·5μ. Giltner; 6·2 Gütig). Neutrophil granules are very fine.

Dog.—Diameter of red cells 7μ. The protoplasm of the polymorphs has a slight neutrophil reaction, and prolonged staining is required to demonstrate distinct granules. The eosinophils have not such a special affinity for eosine as for orange. Mast cells are very rare.

Cat.—Red corpuscles 6·5μ. Granules are present in the polymorphs, but they are exceedingly fine and very difficult to demonstrate. Prolonged staining is required (Leishman, half an hour). The protoplasm tends to stain diffusely in the same tint as the granules. The eosinophils have large ovoid granules. Basophils are found in very small numbers.

Rabbit.—The red cells are poor in hæmoglobin and look anæmic. Average diameter 6·5μ. The polymorphs contain large amphophil granules (pseudo eosinophils). There are also true eosinophils with more densely packed granules. Mast cells are relatively abundant. Wild rabbits generally give higher counts, especially of red cells, than do the domestic variety.

Guinea-pig.—Red corpuscles measure 7·5μ. The polymorphs contain large amphophil (pseudo-eosinophil) granules. The eosinophil granules resemble those of man but they are divisible into two groups which have

somewhat different affinities for dye stuffs. In many of the large lymphocytes there are large vacuoles which may be empty, but which generally contain a homogeneous structure which stains in the tone of the neutrophil granules. These are known as "Kurloff's bodies." They probably consist of a cellular secretion, but have been thought to be parasites.

Rat.—The diameter of the red cells averages 5·8μ. The polymorphs contain rather scanty and fairly large granules. Many of both polymorphs and eosinophils have ring-shaped nuclei.

White mouse.—Red corpuscles measure 5·6μ. The lymphocytes are mostly large, with relatively large nucleus and faintly basophil protoplasm, which contains a good many azurophil granules. The polymorphs are usually stated to be non-granular, but it is possible with prolonged staining to demonstrate fine and very scanty neutrophil granules. These cells are very labile. In the eosinophils the granules are often scanty and may be arranged in patches. Ring-shaped nuclei occur, and unless films are carefully spread the polymorphs, whose granules are often unstained, may be mistaken for lymphocytes.

Hedgehog.—Red cells measure about 4·5μ. The fine basophil granulation of the lymphocytes is very distinct. The polymorphs have distinct granules. There are about 1 per cent. of basophil myelocytes. The granules in some of them are very large.

Fowl.—The great majority of the red cells are oval and nucleated with a long diameter of 11·2μ. A few, however, are round, and in these the nucleus shows a wider chromatin network. These cells correspond to the large nucleated red cells of mammalian blood, and are probably younger cells than those with oval nuclei. Even earlier forms are found in the circulation. These are oval, nucleated, but non-hæmoglobin, containing corpuscles. They are usually smaller than the ordinary forms. They are the youngest type of cell. Again, a few old cells are to be found whose nuclei have disappeared. The white cells are peculiar. There are lymphocytes and eosinophils resembling those of mammalian blood. There are no ordinary neutrophils. Their place is taken by polymorpho-nuclear, cells, whose protoplasm contains a large number of rod-shaped crystalline bodies with a marked affinity for eosin. Basophils are present in considerable numbers.

They are myelocytes with a relatively small, pale nucleus and a pale protoplasm, in which the scanty basophil granules varying in size are to be seen. Blood plates are absent. Small lipoid bodies (hæmoconia) are to be found in small numbers.

Frog.—The red cells have to some extent the same general characters as those of the fowl. The typical cell is flattened, oval, and biconvex, with a thickened equatorial area. There is an oval nucleus. The long diameter measures 22μ. Young forms are also found. These are small, oval cells without hæmoglobin, and round hæmoglobin, containing cells with the megaloblastic type of nucleus. Non-nucleated forms occur with rather greater frequency than in the fowl. The white cells are lymphocytes, polymorphs often with fragmented nuclei and without granules, eosinophils, and basophil myelocytes.

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MENDELIAN ACTION ON DIFFERENTIATED SEX.

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Introductory.—The question of the action of Mendelism in the formation of the human genital tract and on sex is a difficult and intricate one, and I am therefore anxious to present my views clearly and succinctly. This will best be done by my giving my reasons for using Mendelism in this inquiry, by defining Mendelism in its elementary aspects, by stating the theses I hope to prove, and then going on to the proof and the results I believe I have arrived at.

It is usually said that Mendelism does not apply in normal human development, although Bryce has given a sketch of it in his *Embryology*. There is, however, a well-known fact in genital anatomy which seems to me to be a clue, and to indicate that Mendelism is at the basis of fertilisation and development in man—the clue being that in the genital tract of the human female and male there are characteristic sex-organs fully developed, and also traces in each of those of the opposite sex.

This suggested the dominant and recessive gametes and the dominant and recessive condition Mendelism describes in plants, and I therefore began an inquiry into the feasibility of applying Mendelism in relation to the development of the human genital tract and in its action on sex. It is certainly remarkable that in the human female genital tract there should be the epoophoron and its duct, the analogue of the epididymis in the male; and in the male an appendix testis and prostatic utricle, the analogues of the fimbriated end of the Fallopian tube and hymen of the human female. I had hitherto considered these relics as pointing to a past hermaphrodite condition of the organism only, but I hope to show they mean more than this, and that they express conditions under which Mendelian action[1] can take place and be apparent when sex has differentiated.

[1] The fact that when, with Indian corn, a cross of white by yellow is crossed with a white the result is 50 per cent. yellow grains and 50 per cent. white, seems suggestive of the equality of the sexes. I have not been able to apply this fact in this research, but Professor Bateson thinks it important.

Mendelism in its Elements.—I must therefore, in the first place, briefly state what Mendelism, in its elementary aspects, is.

The work of the now famous Prälat of Brünn (1822–1884) was an experimental research into the results obtained by crossing varieties of the eating-pea, possessing respectively distinct and easily recognisable characters, such as end position and lateral position of the flowers, greenness and yellowness in the peas, tallness and dwarfness in the plants, and so on. These varieties bred true to their special characteristic when self-fertilisation, their natural method of propagation, was allowed, and they could be artificially fertilised in a well-known way which I need not detail.

The following is a brief statement of the elements of Mendelism, so far as it bears on the present inquiry:—

When the pollen of a pea-plant with the unit-character of tallness[1] is applied with suitable precautions to the stigma of a dwarf pea-plant, the result of sowing the peas ultimately produced by this cross-fertilisation is a generation F^1 of plants, all tall. The same happens if the pollen is taken from a dwarf plant and applied to the stigma of a tall pea-plant.

Mendel expressed this by saying that tallness is dominant and dwarfness recessive.

Thus,

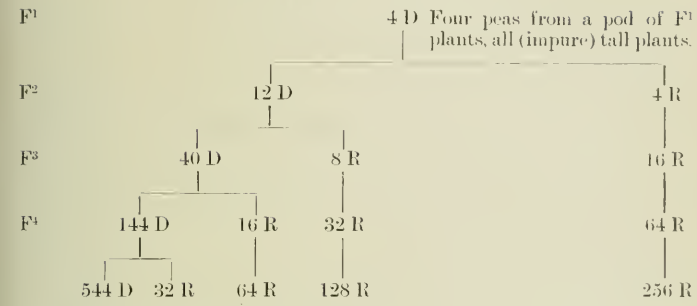
P	D × R
	tall dwarf
	‖
F^1	DR
	impure tall.

When the plants in F^1 are left to self-fertilisation, their natural method of propagation, we get F^2, where the impure talls and dwarfs appear in the proportion of 3 : 1.

The dwarfs now breed true, and the talls give pure talls to impure talls (as shown by sowing) in the ratio of 1 : 2. The following schemes give these results diagrammatically in different ways:—

[1] See p. 6 as to unit-character.

Scheme I. (I. P. Lotsy).[1]



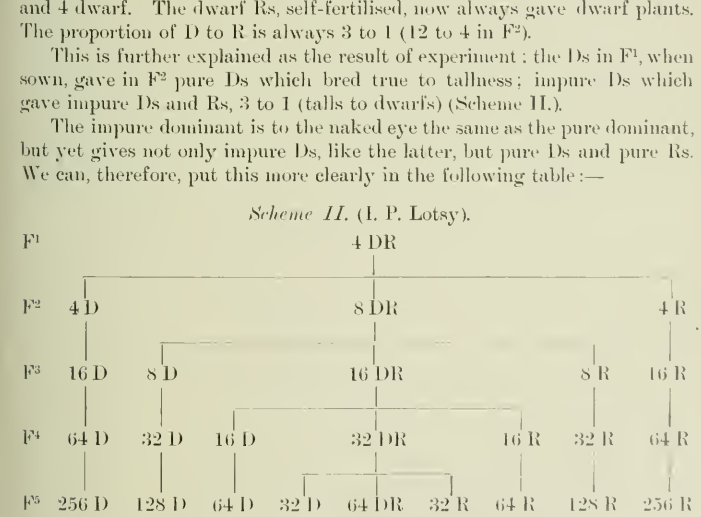
This scheme should be read as follows:—

In 4 D we have four peas of one pod of F^1 where the plants were all impure tall. These four peas sown gave 16 plants, of which 12 were tall and 4 dwarf. The dwarf Rs, self-fertilised, now always gave dwarf plants. The proportion of D to R is always 3 to 1 (12 to 4 in F^2).

This is further explained as the result of experiment: the Ds in F^1, when sown, gave in F^2 pure Ds which bred true to tallness; impure Ds which gave impure Ds and Rs, 3 to 1 (talls to dwarfs) (Scheme II.).

The impure dominant is to the naked eye the same as the pure dominant, but yet gives not only impure Ds, like the latter, but pure Ds and pure Rs. We can, therefore, put this more clearly in the following table:—

Scheme II. (I. P. Lotsy).



[1] 4 D means 4 seeds or peas in one pod of the F^1 generation. The term for plants giving the first crossing is P; the hybrids F^1, F^2, etc. Each pod carried four peas.

This shows that D is to DR to R as 1 : 2 : 1.

The resulting Ds and Rs in Schemes I. and II. are universally the same: D = 544, R = 480; but in the first scheme the pure Ds and impure Ds are not shown separated.

The usual explanation of this is that the unit-characters of tallness and dwarfness are pure and autonomous, not blendable; and that, in 1000 pollen-cells, 500 carry tallness, 500 dwarfness: the ovules have also 500 with tallness and 500 with dwarfness. Thus the gametes are double for each sex of the hermaphrodite pea, and as each has an equal chance of union we may express it thus: $(D+R)(D+R) = D^2 + 2\,DR + R^2$; *i.e.* D : 2 DR : R, the sign of the square being eliminated numerically, as D^2 means the square of the unit-character.

Mendel thus established that—

1. Unit-characters in plants, in this case tallness and dwarfness, (the same holds good for animals as a rule) act as units, *i.e.* independently, and do not blend. Each is supposed to be present pure in the pollen or egg-cells, and in equal proportions of them. The last sentence expresses the theory of gametic segregation.
2. By dominance one or more characters may prevail over, or appear to suppress, others either in the pea or in the plant. Thus, in F^1 the character of tallness appears to dominate that of dwarfness, the recessive element. The same holds good for yellow over green in peas, and so on. The recessive character is, I hold, present unaltered in the gamete determinants, not present in the plants. F^1 is thus made up of impure dominants, but the dominant character is present in the plant, the recessive secluded in the gamete (*c.* p. 36).
3. When F^1 is "selfed" we get in F^2 tallness and dwarfness, 3 : 1; then dwarfness breeds true, but the talls are impure, and when selfed give pure talls to impure talls to dwarfs, as 1 : 2 : 1.
4. The test of a variation proceeding on Mendelian lines is the sowing of seeds, or breeding in animals.

Mendel thus deduced the following facts or laws from his experiments: Dominance and Segregation, which he explained by the theory of gametic segregation. The explanatory theory of gametic segregation seems to me doubtful, but I reserve the discussion of it to the end.

Mendel's supreme merits were as an experimenter, as a lucid and exact interpreter, and, finally, as one who made an exact enumeration of his results and established remarkable ratios.

Having thus briefly explained Mendelism in its most elementary principles, I now go on to state in anticipation of my results, certain theses, but I first define certain terms I purpose using.

The usual terms "gamete" and "zygote" are exceedingly convenient, but we need one for the fully developed product of the zygote—a general term, in fact, for "child," puppy, calf, plant, and so on. The term I propose is "holophyte."[1] I have to thank a classical friend for the suggestion of the term.

Gamete (the spermatozoon or the ovum) has the adjective sex or non-sex, female or male, added as necessary.

Zygote is the fertilised ovum, and I use male and female as descriptive adjectives here too.

Holophyte[2] is the fully developed organism, and may, when necessary, have "adult" and male or female added. The embryo may be termed *Gametozoon*.

I now go on to prove the following theses :—

1. The human fertilised ovum (zygote) is an impure dominant, as in F^1 of Mendel's scheme.
2. The spermatozoon and ovum (the gametes), the agents of fertilisation, are of two types, four in all in number, and unequal in value as to unit-characters. Thus, there are two male gametes of unequal value, and two female gametes also of unequal value. One male gamete contains the male sex-unit-character; one female gamete has the female sex-unit-character. We may thus speak of a male sex gamete and a male non-sex gamete; a female sex gamete and a female non-sex gamete.
3. What we term "fertilisation," giving the zygote, is a crossing or variation either of a male sex gamete with a female non-sex gamete, giving a zygote developing a male holophyte; or a crossing or variation of a female sex gamete with a non-sex male gamete, giving a zygote which will develop into a female holophyte. Thus one of each of the sex gametes contains, *inter alia*, the "determinants" for the characteristic sex of the zygote. What unit determinants I suppose each gamete approximately to have comes out later (see p. 23).

[1] Φυτόν means a plant or animal, and not merely a plant (Liddell and Scott).

[2] Bateson (*op. cit.*, 1909, p. 16, note) says: "In botany the term zygote is usually restricted to the single cell which results from the process of fertilisation, but by a natural extension the word may be used for the individual which develops by somatic divisions from that cell." The use of the term "holophyte" will prevent any confusion that may arise from this extension of the meaning of the term "zygote."

I.—*The Human Zygote is an Impure Dominant, as in F^1 of Mendel's Scheme.*

This I prove by showing that the adult human male and female genital tracts each contains elements unequal in value and function: in the male, potent male and non-potent female components; in the female, potent female and non-potent male components. The potent may be considered as dominant, the non-potent as recessive.

In a paper on "The Nature and Cause of the Physiological Descent of the Testes," I described the human male and female genital tracts, the mamma and Wolffian body, as an associated anatomical unit, under the term of the male and female urogenital unit—for shortness, the male and female genital unit.

I must now analyse the components of each of these units as accurately as possible in regard to their anatomical development and functional activity, and show that they contain elements (1) practically potent and characteristic of the special sex, (2) non-potent and based on the opposite sex-type, and (3) potent but not as fully developed as their analogues in the opposite sex-type.

I begin first with the ***female genital*** unit. This consists of the mamma, Wolffian body and duct, ovarian ligament, round ligament, epoophoron (parovarium), Müllerian ducts, urinogenital sinus, and external genitals.

The *mamma* is well developed and potent. It is normally pectoral and double. Abnormalities may occur, and these have been discussed by several investigators: Leichtenstern, Mitchell-Bruce, Bland-Sutton, Weidersheim, Bateson, Hartung, and others. In early pig-embryos, lateral mammary ridges have been shown to exist on the anterior abdominal surface, the "milk-ridges": these are beaded and disappear after a time, and are not the *Anlage* of the permanent teats. These milk-ridges have been found in a human female embryo (15 mm.) in a less complete condition. They were most marked above, ran caudally for 1·5 to 4 mm., and only slightly backwards. They are probably phylogenetically related to the epidermis ridges on the anterior abdominal wall, in the lower end of which, as I have shown in the Marsupials, the scrotum develops. In relation to these milk-ridges, if not from them, the mammæ develop, and this explains supernumerary teats and mammæ in the human male and female. These have been found in the inguinal region, and it is of special interest to note that a labial mamma has been described in the human female by Hartung. I do not summarise our knowledge on this subject, but must draw attention to Dr Florence Sabin's account of the development of the superficial lymphatics in the anterior abdominal wall of the pig-embryo, where she shows that there are for this

region four sources of origin of the lymphatics, from veins approximately at the extremities, upper and lower, of the "mammary line." The importance of this lower origin, as to the growth of the gubernaculum and round ligament, I have already emphasised in a previous paper.

In the human female holophyte, then, the mammæ are fully developed, double and pectoral; they are potent, that is, functional, during adult life.

The *Wolffian body* has disappeared in the genital unit, but has left behind, *inter alia*, the round ligament and ovarian ligament, both well marked and very evident.

The *ovarian ligament* is the analogue of the caudal ligament of the testis, and, unlike it, persists, *i.e.* is potent or evident, and probably acts in the adult female as a ligament of the ovary.

The *round ligament* is the analogue and homologue of the developing "gubernaculum" of the male. Its upper end is at the junction of Fallopian tube and uterus, and has the uterine attachment of the ovarian ligament opposite it and on the other side of the tube. It is a persistent part in the genital tract, and corresponds exactly to the gubernaculum while the testes and Wolffian bodies are in the abdomen and high up, and the inguinal canal has been developed but does not yet contain the testes. It is thus, to put it shortly, the analogue and homologue of the developing gubernaculum, prior to testicular descent. It does not go on to complete development or involution as the gubernaculum does, and is thus semipotent, partially developed.

Between the layers of the broad ligament in the space between the ampulla of the Fallopian tube, ovary, and ovarian fimbria, lies a structure, normally present in a female holophyte, known as the *epoophoron* or *parovarium*. This consists of a series of communicating tubules running at right angles to the Fallopian tube, and communicating at their Fallopian tube ends with a longitudinal duct. The latter is prolonged as a single tube, and ends near the junction of the tube and ovarian ligament and opposite the origin of the round ligament. These parallel tubules correspond to the epididymis of the male, and the longitudinal tube to the duct of the epididymis, that part of the vas deferens which ends at the globus minor. Where the globus minor joins the straight portion of the vas, the gubernaculum takes its origin; and thus the permanent portion of the Wolffian duct between the layers of the broad ligament in the female equals the duct of the epididymis. The epoophoron and duct thus equate with the epididymis and its duct.

The epoophoron and its duct are non-potent, imperfect in development and structure, and the analogues of potent male sex structures.

The rest of the Wolffian duct is said not to be represented in the female,

but in reality is so, in part. I have shown that the Müllerian ducts form only the upper two-thirds of the adult vagina, that the lower third is formed by the upper part of the urinogenital sinus, and that the hymen is formed by the coalescence and breaking down at the third and a half month of fœtal life of two epithelial bulbs (the Wolffian bulbs) arising from the lower ends of the Wolffian ducts, which, as Kollmann has shown in the human embryo, have an ectodermic origin. These, and an active ingrowth from the urinogenital sinus, make the hymeneal entrance. The double bulbs have been also found by Dr Wood-Jones, and in a recent paper on the development of the hymen in the white rat, Kempe states that the hymen is not formed from the Müllerian ducts, but from an epithelial mass below it. The summary of his views, as given in Schwalbe's *Jahresbericht* (xxi. 632), is as follows: "Die distalen Enden der beiden Müllersehen Gänge werden durch eine Epithelquerbrücke vereinigt und diese Epithel Brücke sieht Kempe als etwas selbständiges an, und zwar als aus der Bodenlage eines ehemaligen vaginal Blindsackes entstanden."

The special point to be noted, then, is that the Wolffian ducts persist at their lower ends to form the hymen. The Wolffian body and its duct are thus, after the second month, non-potent.

The *urinogenital sinus* persists in the female in its lower portion as part of the vagina, with the exception of a small longitudinal section forming part of the urethra: the upper portion of the urinogenital sinus completes the urethra. The upper segment of this lower part of the urinogenital sinus makes up the lower third of the vagina, and the rest of it below forms the vestibule. The "vestibule" runs up as far as the clitoris in front, and down to the fossa navicularis, where the hymen and fourchette blend. It passes laterally to the basis of the labia minora. The urinogenital sinus is thus potent.

The *labia majora* are separate and solid, and the round ligaments end in them. The labia majora correspond to an early arrested stage of the scrotum, and are in two halves: they are thus potent, but incompletely developed as compared with the scrotum.

In the *glans clitoridis, vestibule, and labia minora* we have permanently what is an arrested stage in the male, and what, if it persisted in the male, would be termed "hypospadias."

The *corpus spongiosum* is represented in the female by a vertical mesial ridge in the vestibule. Thus, in the external genitals of the adult female, the phallus is represented by the imperforate glans, the prepuce is defective, the corpora cavernosa are less developed than in the male, and there is no equivalent to the prostate, except perhaps Skene's tubules.

The external female genitals are accordingly potent, but less fully developed than their male analogues. They form really a link between

potent and non-potent elements, resembling the latter in being segmentally defective but differing in possessing a perfect anatomical structure.

The *ovaries, Fallopian tubes, uterus, and vagina* are fully developed and "potent."

An adult human female has therefore in her genital unit:—

1. A fully developed, evident segment, characteristic of her sex: it comprises mammæ, ovaries, ovarian ligaments, Fallopian tubes, uterus, vagina, hymen, urinogenital sinus.
2. A part—viz. the round ligament, the labia majora and minora, and the glans—potent, normal in structure, less fully developed than in the male, but always analogous to a definite continuous segment of the corresponding male organs.
3. A part, normally present, made up of developmental portions equivalent to corresponding fully developed organs in the male, but less perfect in structure and not equally functional: these comprise the epoophoron and the upper portion of the Wolffian duct.

It must be noted that in 2 and 3 the deficiently developed or incomplete organs are all precise and exact in their anatomical conditions. The epoophoron corresponds exactly to the epididymis and the duct of the epididymis; and so on, as I have already indicated.

I may therefore sum up under this head as follows:—The female genital unit has elements that might be classified as "potent" and "non-potent." The "potent" are: mammæ, ovaries, ovarian ligament, tubes, uterus, vagina and hymen, urinogenital sinus, and lower end of Wolffian duets, external genitals, round ligament; the "non-potent" are: epoophoron, upper segment of Wolffian duct.

The potent here are perfect and characteristic of the female sex: the non-potent are incomplete and less developed portions of the "male" "dominant" organs, but are definite portions of these. The potent may be spoken of as dominant, the non-potent as recessive.

To appearance, then, the female genital unit contains "potent" organs, but when we analyse them carefully we find a part only potent; another part, well defined, but much less developed—the epoophoron and its duct, generally described hitherto as developmental debris, or as a phylogenetic reminiscence of a supposed remote hermaphrodite phase in the vertebrata, or at any rate in the invertebrates—is accurately termed "non-potent" or recessive, is a less developed male element, and is of the greatest scientific importance.

I may note finally under this head that the potent part in the female genital unit is less fully developed than in the corresponding male unit: the non-potent elements are more numerous.

The ***male genital*** unit has the mamma pectoral and double, but, compared with that of the female, much less developed. Mammary repetitions in man are less marked and less abundant than in the female. They do not occur below the navel, and there never has been any scrotal teat or mamma described corresponding to the labial mamma of Hartung. The scrotal area is none the less a mammary one.

The persistence of the mamma in man has been explained by supposing it to be a survival of the time when the male aided the female in suckling their young. This may be so, but I would also associate its permanence with the fact that, as the gubernaculum is persistent and unaltered in man, the area into which it always passes, a mammary area, is endued with a like persistence. The mamma in the male is "non-potent," evidently.

The *gubernaculum* in the male joins the epididymis by means of the caudal ligament of the testis, and has thus, at first at any rate, no direct insertion to the testis, but to the Wolffian duct, in its early development. It thus connects the testis indirectly to a mammary area, and in this way influences the descent of the testes. In the male the gubernaculum is a "potent" structure, but its power is transient. It practically disappears after the testis is lodged in the scrotum. It thus contrasts with the round ligament and the ovarian ligament, as these are well marked and permanent in the female. The gubernaculum, however, passes through a more perfect development and function than the round ligament, which remains at a fixed early stage of its development.

The gubernaculum is thus a "potent" but transient part of the genital tract.

The *testes*, *phallus*, and *scrotum* are all "potent."

The *prostatic urethra* is derived from the urinogenital sinus. On its posterior wall the prostatic utricle with the openings of the vasa deferentia can be seen, and it most probably normally represents the hymen only. My view on the development of the hymen explains this exactly. Just as the hymen is developed from the lower ends of the Wolffian ducts, so is the male hymen from the lower ends of the vasa deferentia. I have also found a bulb of epithelium of a squamous vaginal type in the prostate of a four months' foetus, lying in the line of the vas deferens, and although the various stages have not been traced, it probably is concerned with the formation of the prostatic utricle. The prostatic utricle is evidently less developed than its analogue the hymen.

The *prostate* in the male is well developed and potent as compared with the Skene's tubules of the female; and it is advisable to consider the prostatic urethra as being also potent.

The *ducts of Müller* are represented in the male by a trifling part

corresponding to the fimbriated end of the Fallopian tube, and, as a rule, in no other way: and the latter is finally the appendix testis of the adult.

The ducts of Müller and the hymen are thus "non-potent" in the male.

There are some trifling relics, the organ of Giraldès or paradidymis, in the male to be noted; they are Wolffian, but are of no special significance.

In the male and female genital units there are thus potent (dominant) and non-potent (recessive) elements, which may be arranged as follows:—

Male Potent (Dominant):	*Female Potent* (Dominant):
Testes.	Ovaries.
Phallus.	Ovarian ligament.
Prostate and prostatic urethra.	Round ligament.
Scrotum.	Müllerian ducts.
Wolffian ducts.	Urinogenital sinus.
Wolffian bodies.	Hymen.
Gubernaculum.	Mammæ.
Caudal ligament of testis.	External genitals.
Male Non-potent (Recessive):	*Female Non-potent* (Recessive):
Mammæ.	
Prostatic utricle.[1]	Epoophoron and rest of Wolffian bodies.[1]
Fimbriated ends of Fallopian tubes.[1]	
Paradidymis.	

I have not discussed the question as to whether the pronephros is represented among the non-potent elements in the female. I have found its traces in the upper end of the Wolffian body of a four to five weeks' human embryo. One interesting point is, however, the question as to whether the duct of Müller is not the representative of the pronephric duct. This would make it analogous to the vas deferens and establish an interesting analogy and homology, but the evidence as to this is as yet incomplete. Whatever is the correct view, it does not affect the general summary of recessive elements.

When I began this inquiry, I first classified the elements in the genital tract as potent and non-potent, and then as dominant and recessive: this seemed to me quite tenable. I also thought that the gametes might be considered as dominant and recessive, each with the dominant or recessive unit determinants only (see p. 31). This, however, proved to be untenable, and I therefore speak of gametes of unequal value. The reason for this will be seen subsequently, but I may say that in the dominant or sex-

[1] I select these as the typical recessive elements in their respective genital tracts.

gametes I hold the characteristic potent and non-potent sex determinants along with somatic determinants are placed, and that in the other—the *non-sex gamete*—no sex determinants are normally represented, only certain somatic ones. This view, therefore, is not in agreement with the Mendelian theory of gametic segregation, according to which the dominant and recessive elements should be in separate gametes.

I now go on to apply Mendelism, and in doing so to use in part Weismann's terminology. Weismann has clearly shown that the holophyte must be represented causally and exactly in the zygote. His nomenclature is most convenient and precise. In the zygote, the determinants—the causes—apparently in the chromosomes, are related to the determinates —their results—in the holophyte, causally. I do not use all Weismann's nomenclature, as this is unnecessary in the present connection. Its use has been satirised as a "portmanteau theory": you get out what you put in. I have not found it so. It is a most convenient means of expressing one's ideas and researches on these points, and its use is most suggestive and leads to useful extensions of knowledge. Not only are the determinants and determinates related causally, but we may speculate further and say that each holophyte cell, a microscopical determinate, contains in it a share of the determinants, and is thus the "biophor" of Weismann, inconceivably minute, moulding the life and career of the cell. This view is really an inversion of Darwin's pangenesis. Darwin supposed that each body-cell gave a minute determining particle of itself—a gemmule—to the germ-cell, a gemmule of determinate to the determinant, thus making acquired variations transmissible: Weismann, on the other hand, makes the biophor go to the cell, the determinant particle to the determinate, during development, and on this theory acquired peculiarities are not transmissible—the correct view, so far as our knowledge goes. (See, however, Lotsy: and Eimer's *Organic Evolution*, transl. by J. T. Cunningham.)

Mendel supposed that the unit-characters in the holophyte were represented in the gametes, thus anticipating Weismann's view, and I therefore speak of the unit-determinates in the holophyte and of the unit-determinants in the zygote. The latter are autonomous, however, *i.e.* transmissible only as such: and this is one of the great results of Mendel's experiments.

I also use Weismann's term, Id, as meaning the aggregate of unit-determinants in the zygote necessary for the determinates of the holophyte.

If, then, we take up the genital unit and apply Mendel's and Weismann's views and terminology to it, we may say that the male and female holophyte are made up of somatic unit-determinates and genital unit-determinates. It is with the genital unit-determinates I am now concerned, and

in them we have, according to the views already expressed, potent and non-potent unit-determinates as well as less fully developed yet potent ones. As Nägeli and Weismann have shown that what is in the holophyte is represented in the zygote, we must thus have in the zygote, unit-determinants of unequal value making up the genital unit-determinants. I do not discuss here whether the somatic determinants can be so divided; they most probably can, but it does not make any difference in my argument. The presence of genital unit-determinants, potent and non-potent (dominant and recessive), defined and limited as at p. 28, and of unequal value in the zygote, makes it an impure zygote of the first generation or F^1. This being the case, I go on to discuss the application of Mendel's "gametic segregation" and "dominance" to the further solution of the question, *i.e.* to fix how the "gametes" produce this "impure dominant," so far as their number and functional powers are concerned.

II.—*The Spermatozoa and Ova, the agents in fertilisation—the male and female gametes—I suppose to be four in number in all, to be unequal in value;* i.e. *we have two spermatozoa of unequal unit-determinant value, and two ova of unequal determinant value; only one of each contains the sex determinants, dominant and recessive: the others contain only somatic determinants.*

The human zygote is the result of the union of the male and female gametes. How may these produce an impure dominant zygote? The usual opinion is that the human zygote is the result of the union of a certain portion of one male gamete, the male pronucleus, with a certain part of one female gamete, the female pronucleus. This, however, is generally held to involve the result that we get a zygote which is at first "bisexually" capable, *i.e.* male and female, and becomes monosexual during its subsequent and early development. This cannot be the case, as we know, *inter alia*, from an elementary consideration of like-twins[1] long ago made by Schultze. Like-twins can only be derived from a male or female zygote, as is clearly shown in conjoined twins, which are always of

[1] When twins are of different sexes they come from two zygotes. Conjoined twins are always of the same sex (Siamese twins, two-headed nightingale, etc.). No exception to this is known. Like-twins (or triplets in rare cases) are almost exactly alike and, to the non-maternal eye, undistinguishable, and must have arisen by a primary equal division from one zygote. Twins of the same sex, and differing as ordinary brothers and sisters do, arise from different zygotes. We may therefore put it this way:—Twins are enzygotic or duozygotic. Like-twins are always Enzygotic; twins, boy and girl, are always Duozygotic, but twins of the same sex, and differing as ordinary brothers and sisters do, may be duozygotic. The enzygotic have, of course, a single chorion; the duozygotic, a chorion round each amniotic cavity—monochorionic and dichorionic twins respectively.

the same sex. Sex, I believe, is determined and unalterable *when the zygote is formed*; no change is possible as to sex after that, and all the views as to environment, or food, or emotion, and so on, in determining sex, are irrevelant, notwithstanding the current views as to the production of sex in certain insects. In a useful summary on this point, Beard, in an able paper on the Determination of Sex, has shown that, in many invertebrata, the spermatozoa are dimorphic, and that ova also may be dimorphic, although fewer instances have been recorded. In man, dimorphic spermatozoa have been described by von Widersperg.[1]

Beard considers that in the metazoa there are "three sorts of functional gametes—spermatozoa, male eggs, and female eggs. A fourth sort, giving two kinds of functional elements in the male, only appears as an abortion, futile or useless sport."

To produce the human heterozygote (or impure dominant), two varieties (this word is exactly correct, as we shall see) of male gametes and two varieties of female gametes seem necessary. The male and female gametes must be of unequal value respectively, and one only of each sex must contain the potent and the non-potent sex-determinants. In this way we get approximately 50 per cent. of each sex, as actually happens.

Now, I further suppose that in fertilisation the male sex gamete unites with the female non-sex gamete to form a male zygote; while the male non-sex gamete would unite with the female sex gamete to form a female zygote. The male zygote, which develops into the male holophyte, thus has the determinants of the male genital unit (potent male and non-potent female): while the female zygote has the determinants of the female genital unit (potent female and non-potent male). Thus the male holophyte has potent male and non-potent female elements in its determinates, and the female holophyte has potent female and non-potent male determinates also.

It may be said, however, why may not a sex male gamete unite with a sex female gamete? The practical answer is that in no normal or abnormal human holophyte hast ever both potent male and potent female determinates been discovered. A like answer can be given to a supposed union of non-sex male and female gametes. A theoretical reply to the question can be given, but this I do not take up at present.

We now see why we have in the genital tract not simple potent sexual units, but each with a potent element proper to its sex and a non-potent element representing the opposite sex. *It is the result-expression in the*

[1] McClung has found that in certain insects half the sperms have an accessory chromosome and half are without it. Those which have the accessory body give with the ovum females. Thus the chromosomes in the sperm-cell are either n or $n-1$, those in the female cells n, so far as is yet known.

determinates of the holophyte of the unequally allotted determinants in the gametes crossing or fertilising, the potent and non-potent behaving as conjoined dominant and recessive elements.

We have now to consider the changes in the germ-cells and sperm-cells prior to the formation of the gametes, and this raises the fundamental question of the origin of these.

It has now been shown in regard to the former in a large number of species, although by no means generally accepted, that the primitive germ-cells are not somatic in their origin, *i.e.* do not in mammals arise from the germ-epithelium. They are really set apart first as the primitive germ-cell mass in the earliest division of the zygote for the purpose of carrying on the "stirp" or "strain" of the future generations. They are not modified, so far as we know, by the subsequent body-changes in any way. The body or "soma" is their trustee, as Galton put it, and cannot "intromit," as Scottish lawyers say, with the germ-cell estate. The germ-cells are entailed, to vary the simile, but not the somatic ones. This fact of the setting apart of the primitive germ-cells for the production of gametes and their continuity I shall term the "Owen-Weismann" law, for the following reasons. Owen, in 1849, stated that "not all the progeny of the primary impregnated germ-cells are required for the formation of the body in all animals: *certain of the derivative germ-cells may remain unchanged and become included in that body which has been composed of their metamorphosed and diversely combined or confluent brethren: so included, any derivative germ-cell, or the nucleus of such, may commence and repeat the same processes of growth by imbibition and of propagation by spontaneous fission as those to which itself owed origin*; followed by metamorphoses or combinations of the germ-masses so produced which concur to the development of another individual in which the secondary germ-cell or germ-mass was included" (*op. cit.*, pp. 5 and 6). Although he afterwards withdrew this view, he still retains the credit of the great fact. Since then many notable workers—Eigenmann, Galton, Weismann, Jäger, Rauber, Boveri, Nussbaum, Balbiani, Beard, and others—have elaborated this observation in great detail, and Weismann has founded his theory of the continuity of the germ-plasm. This is best illustrated by a quotation from Weismann's great *Apologia pro opere suo*: "This splitting up of the substance of the ovum into a somatic half which directs the development of the individual, and a propagative half which reaches the germ-cells and there remains inactive, and later gives rise to the succeeding generation, constitutes the theory of the continuity of the germ-plasma, which I first stated in a work which appeared in the year 1885" (*The Evolution Theory*, Thomson's translation, vol. i. p. 411).

Balbiani's description of what happens as to this point in the well-known fly, *Chironomus*, is very clear:—"Before the segmentation of the egg had at all advanced, before what embryologists had called the blastoderm was more than incipient, two cells were observed to be set apart externally (these had nothing to do with the polar bodies seen in most ova at the maturation). The development proceeded apace, but the isolated cells took no share: they may be presumed to have retained intact the characters which they received when first divided off from the ovum. At a certain stage, however, the isolated cells sank inwards, took up an internal position, became the rudiments of the reproductive organs. Here then, at an early stage, before differentiation is marked, the reproductive cells are set apart. They must, therefore, preserve much of the character of the parent ovum, and hand on the tradition intact by continuous cell generation to the next generation" (quoted in *Evolution of Sex*, revised edition, Geddes and Thomson, p. 98).

Beard especially has worked out this early zygotic origin of the germ-cell mass, and the continuity of the germ-cells, with great care in his valuable papers. I cannot give details of his many researches, but the following gives the parts bearing on the present inquiry. Beard has shown that "on taking an early Elasmobranch embryo and staining this with certain reagents (osmic acid or Heidenhain's iron hæmatoxylin) the germ-cells within it are rendered so prominent that one can even count them. These germ-cells are seen to be cells of large size, about ·02 mm., whereas the largest somatic cells do not exceed 0·012 to 0·014 mm. Their products when they do divide are termed secondary germ-cells. . . . From the counting and tabulation two results among others stand out prominently. These are that the number of primary germ-cells is a constant quantity for the species, and that during long periods of the development—in *Pristiurus* in embryos from 4 mm. to 20 mm., in *Raja* from 6 mm. to 42 mm.—no single embryo has ever all its germ-cells in the normal position. . . . This is certain, that is to say, it can be established that the germ-cells arise before there is any embryo, and that on its formation they migrate into it along the connection between embryo and yolk-sac, the yolk-stalk. . . . Owing to these migrations from the yolk-sac and blastoderm, it never happens that all the germ-cells find their way into the normal position, the germinal nidus or its future site. Thus sometimes they may be found in the head, head somites, brain, and gill region, the skin, the trunk or head, the pericardium, the liver, the body cavity, etc."

The significance of the parts of these observations as to teratomata will be considered afterwards.

An objection sometimes taken is that the Owen-Weismann law has not

been demonstrated in human development. It must be admitted that the evidence is as yet of the scantiest, but this is due to the difficulty of obtaining specimens. It is satisfactory to know, however, that a beginning has been made, as Ingalls has demonstrated and figured, in a 4·9 mm. human embryo, primitive germ-cells, in or below the cœlomic epithelium, near the root of the mesentery and on the mesial side of the Wolffian fold in the region of the first five rump-segments. ("In oder unter dem Coelomepithel nähe der Wurzel des Mesenteriums auf der medialen Seite der Urnierenfalte, finden sich im Gebiet der ersten fünf Rumpfsegmente mehrere grosse als Urgeschlechtszellen zu bezeichnende Zellen," *op. cit.*, S. 547.)

The zygote is formed by the crossing of a male sex gamete with a non-sex female gamete, or by that of a female sex gamete with a non-sex male gamete, in both cases the crossing gametes being of unequal value.[1] Thus formed, it first divides into two parts. Each of these is a zygote, but the one is the primitive germ-cell mass, from which the primitive germ-cells will arise and then the gametes. I have already spoken of the determinants of the zygote and the determinates of the holophyte, related as cause and effect, and I now wish to use another term of Weismann, viz. the Id, and to repeat its definition (p. 14) as the group of determinants necessary for producing the determinates of a holophyte.

These two parts of the zygote are essentially the same, but there is a difference. The primitive germ-cell mass necessarily contains thousands of Ids, but the other half of the zygote, which is to form the "soma" of the holophyte, necessarily contains only one Id, without germ-cells, as a holophyte needs only one Id. When this half develops into the "soma," the primitive germ-cells, with Ids probably differing from the somatic Id in somatic determinants, derived without reduction from the primitive germ-cell mass, travel through the blastoderm and along the yolk-stalk, and ultimately, as I have said, mobilise on the Wolffian ridges. F. A. Woods has discussed the question how far disproportionate growth might account for this germ-cell journey, and it may account for some changes; but an amœboid or travelling power must apparently be a property of the germ-cells. When the germ-cells thus reach the germ-epithelium on the Wolffian bodies, the embryo is once more joined up, *i.e.* the results of the first primitive zygote-division are combined. These two parts have, as it were, marched separately, and now unite so as to give an embryo capable of growth and ultimate propagation. It thus contains one developing Id, and in the ovary or testis thousands of Ids.

These two halves of the zygote are thus essentially the same, but the one is phylogenetic in its nature and entailed; the other ontogenetic, at the

[1] The determinant value of each gamete is considered at p. 28.

disposal of the individual; but this individual holds his or her sexual cells as an entailed estate of which only a certain use, *i.e.* the propagation of the species, is allowed, and where the gametes do form a fresh zygote, the primitive germ-cells are once more handed on as a part of the new zygote, practically unaltered as already described. This must be considered a great fact in heredity, and shows the importance of "race" or "strain."

We must now consider the changes that go on in the germ-cell mass subsequent to its formation. These may be considered in essentials to be: (*a*) Non-reduction changes of the germ-cell mass, so that the primitive germ-cells (secondary germ-cells of some authors) are formed; (*b*) spermatogenesis and oogenesis, so that the gametes are formed probably by the throwing off of the polar bodies, *i.e.* reduction.

(*a*) *Non-reduction Changes in the Germ-cell Mass, so that the primitive germ-cells are formed.*—The primitive germ-cell mass is formed according to the Owen-Weismann law, and it thus logically follows that it is *zygotic in its nature*. This is a most important consequence of this law, and has not been followed out by any observer so far as I am aware.[1] The changes that take place in a primitive germ-cell mass first are those of its division or multiplication into the primary germ-cells, and these, as no reduction takes place, are also zygotic.

As a zygote *in posse* there must be further changes in the primitive germ-cell to take away the power of zygotic differentiation into a gametozoon and ultimately into the holophyte, and this is, I believe, the object of the reduction of the chromosomes (spermatogenesis and oogenesis respectively) forming the gametes.

(*b*) *Spermatogenesis and Oogenesis,*[2] *so that the gametes are formed.*—The great changes here are the reduction of the chromosomes of the primitive germ-cell so that each gamete has only half the number of chromosomes of the primitive germ-cell or of a somatic cell. The determinants of the primitive germ-cells, somatic and genital (dominant and recessive), are thus divided between the gametes, but not, I hold, so that each gamete contains only dominant or recessive determinants; and then by a crossing of dissimilar gametes a fresh zygote is formed. This, I urge, may be the significance of the reduction of the chromosomes—not an equal division into maternal and paternal determinants of heredity, as Weismann has put it, but an unequal separation of the primitive germ-cell into sex and non-sex gametes.

If these sex and non-sex gametes are thus divided, the question arises,

[1] It may be that Owen's statement implies it (*v.* p. 17), but his meaning is obscure.

[2] These are very complex, especially spermatogenesis, owing to the ultimate complicated structure of the spermatozoon.

Can we find how the determinants are quantitatively allotted? Which determinants go to the non-sex gamete, and which to the sex one? Before considering this I wish to make use of a generalisation of Przibram's as to "fertilisation." Przibram states in his most valuable monograph on *Experimental Zoology: Embryogeny*, Part I., p. 30, that:—"There is a normal association between division of the centrosome, formation of centrospheres, astrospheres, division of the nucleus and the yolk (cell-body), and perhaps also progressive differentiation of the cleavage cells; but we must not conceive of this series in such a way as to suppose that one member is the determining cause of the next, but rather that all the processes are called forth in succession by a common cause, and then provide jointly for the typical development."

We may thus say that when the gametes are formed the centrosome and nucleus are probably divided between the gametes, and when these are so divided the gametes lose the power of zygotic differentiation into a holophyte. Each gamete has lost the power of zygotic development, but a crossing of gametes normally restores this—one of the objects of fertilisation.

I wish now to ascertain how the determinants of the primitive germ-cell are divided between the gametes, a question that lies at the base of heredity. I attempt this in a novel way—not exact as yet, but approximately so. I take the most fully developed cases of what is termed a teratoma,[1] *i.e.* a solid dermoid tumour, of the ovary or testes, with holophyte determinates, normal in their development and arising from all the three germ-layers, as Wilms and Okhubo have shown; and regarding these, for reasons given afterwards, as arising from an aberrant gamete, I reason back from the determinates of the teratoma to the determinants of the aberrant gamete from which I suppose it arises. If I settle the determinants of one gamete, then those in the other gamete follow. I must therefore discuss (1) the structure of the teratoma, (2) the way the teratoma arises, (3) the determinants approximately present in each of the gametes.

(1) *The Structure of the Teratoma.*—Under certain conditions, as yet

[1] I may say, for the non-medical reader, that in the ovary especially, and in the testis, are quite frequently found tumours with definite and normal body-elements—hair, teeth, mammæ, brain-tissue, bodies, bowel, etc. When in the ovary these structures are present, along with creamy-like fluid, it is called a "dermoid" tumour of the ovary; when distinct parts are found so advanced that it is part of an embryo, it is called an embryoma or teratoma. Such a structure may be practically solid; chorionic epithelium may also be present (epithelium from the early outer covering of the zygote)—and these may give rise to malignant conditions, chorio-epitheliomata, analogous to those that we now know so frequently follow the hydatid mole pregnancy in women. Dermoids may be found also in other parts of the body, and the explanation of this will be given subsequently. The body tissue found in embryomata is quite normal.

incompletely known, an imperfectly reduced gamete (probably), or according to others a primitive germ-cell, may develop in the ovary into a partial embryo, the embryoma of Wilms or teratoma of other authors. The remarkable fact about these ovarian teratomata is that in their most advanced examples they consist of parts of normal determinates of the holophyte, viz. body-trunk (upper part), imperfect limbs, parts of brain and spinal cord, respiration-tract, mamma, labia majora, intestine (*Darmschlauch*), rudiment of eye. These teratomata are thus not merely ectodermic products (in fact, these do not predominate), but contain derivatives of all the germ-layers (Wilms). Thus Shattock, in his Erasmus Wilson Lecture, has described a very perfect specimen, probably the most developed teratoma known, which consisted of a trunk furnished with two lower limbs, and a peritoneal cavity containing a blind coil of intestine. The skeleton comprised a short rudimentary spinal column with a pelvic girdle, and in each of the lower limbs there was a long osseous element filled with fatty marrow. Between the limbs were two distinct labia, bounding a blind depression, and behind these a perineal raphe. It had thus in the main the structures assignable to somatopleure, as I shall presently explain.

What is *not* represented in the ovarian teratomata is probably the small intestine, and *the characteristic genital organs have certainly not yet been found represented.*

Thus we may regard the holophyte or adult as divided into two great groups of determinates, the one comprising the somatopleuric part of the body—head, trunk, mamma, limbs, brain, and spinal cord; *plus* mouth and œsophagus: small portion of bowel, bladder: bronchial tract, heart-muscle, spleen, cord: lymph-glands. The other would contain the splanchnopleuric part of the body, or rather the pleuro-peritoneal cavity (cœlom) and some of its contents, viz. small intestine at any rate, and genital organs.

Thus probably the best statement as to the relation of these two body-divisions to the germ-layers is that the former is in the main somatopleuric, the latter splanchnopleuric. There is probably a common area where these blend.[1] The analysis of the determinates in an ovarian teratoma is undoubtedly difficult. I rely most on the analysis of naked-eye specimens such as Wilms' and Shattock's: the microscopical results are also valuable, but are more difficult to link with organs, *e.g.* in the case of unstriped muscle.

The absence of dominant genital determinates especially leads me to suggest the development of ovarian teratomata from an imperfectly reduced primitive germ-cell, from a non-sex gamete and not from the unreduced primitive germ-cell. This is, however, more fully taken up afterwards.

[1] Somatopleure = ectoderm *plus* half of split mesoblast; splanchnopleure = entoderm *plus* half of split mesoblast. The split cavity is pleuro-peritoneal.

Okhubo, in a very comprehensive consideration of testicular embryomata, has shown that they also contain representatives of all the germ-layers and microscopic elements of almost all organs (see, however, *ante*, p. 22) and of chorio-epitheliomatous tissue. They do not contain determinates of the dominant genital tract. His own cases were eleven in number, and all were examined thoroughly. He also has collected from the literature on this subject 107 cases, and analysed the results. I hope to show that the testicular teratoma is derived from an imperfectly reduced non-sex male gamete, just as the ovarian teratoma is derived from an imperfectly reduced ovum. I may say in passing that a particularly searching examination of all teratomata as to the actual organs and tissues represented, and to what extent, is imperatively necessary, and would be likely to afford valuable results.

The question now arises as to how far this division of the holophyte into somatopleuric and splanchnopleuric components can be linked with the gametes. It can, to a certain extent, I believe, and the clue to it is in this, that the characteristic genital unit-determinates have not been found in the ovarian or testicular teratomata. This leads one to the possibility of the female sex gamete containing the determinants for the splanchnopleuric segments and sex-organs of the holophyte; the non-sex one those of the somatopleuric portion. I cannot suggest as yet anything precise as to the segregation of the viscera-determinants in the gametes.

The same holds good for the male sex gamete and male non-sex gamete. Thus, as the result of crossing of gametes as already suggested, the female holophyte would have the splanchnopleuric segment and genital organs from the female sex gamete, *i.e.* (in the main) its sex and splanchnopleuric determinates; its somatopleuric determinates (in the main) from the non-sex male gamete.

In the same way the male holophyte would have its splanchnopleuric and genital unit-determinates from the male sex-gamete; the somatopleuric segment from the non-sex female gamete.

This in the main, put popularly, would make the human female follow the male parent in mental and muscular body development: from or through the mother she would get sex, digestive, and excretory organs in part. The human male would in like manner get his mental characteristic and muscular body development from or through the mother; his sex, digestive, and excretory organs in the main from or through the father.[1] This puts the matter broadly. It is evident, however, that such a conception is not always sharply realised, and that an interchange of determinants does take

[1] One often sees the average-size mother with a tall father bear a son tall like his grandfather. In other cases the son is tall like the mother.

place, not in exact accordance with this idea. Before the polar bodies are thrown off, however, and the chromosomes exactly divided, there is a spireme or continuous arrangement of these chromosomes, and it is here, from or in connection with this stage, that such changes may take place, before the exact and equal division of the chromosomes occurs. What I have advanced implies that in the reduction of the primitive germ-cells there is no equal division of paternal and maternal characteristics, but an unequal segregation of determinants in the two varieties of ova and spermatozoa, and then a crossing of a somatopleuric (non-sex) and a splanchnopleuric (sex) gamete of each variety to give a zygote with a variation of determinants. There is thus a rearranging of autonomous unit-characters—an unequal segregation to form the gametes, *i.e.* these are not pure as to dominance and recessiveness: a crossing of unequal gametes to form the zygote; but the unit-characters do not blend, are not diluted fractionally, but are present, if not always evident, in each generation, unless they breed true to certain of their unit-characters.

In connection with this result, at which I arrived independently, I may quote a remarkable illustration of it in a passage in Przibram's most valuable work, as follows (*Embryogeny*, p. 62):—"The normal relations of the several blastomeres of *Ascaris* to the later organs and portions of the body are described by Zur Strassen in the following account based upon his own results and those of Boveri. The egg of *Ascaris megalocephala* is divided by the first cleavage into cells lying one above the other, which differ fundamentally both in their prospective significance and their mode of development. From the smaller cell which lies beneath, are produced the greater number of the organs, the sexual system, the mesoderm, the whole of the digestive tract, and a part of the body-wall. This lower cell-group, which is confined to the ventral side and the posterior end of the animal, divides with a very irregular rhythm, but all its descendants are sharply characterised, even in the later cleavage stages, as regards their mutual position and the nature of their nuclei. On the other hand, the larger cell lying above furnishes a single structure, the 'ectoderm,' which develops at a uniform rate. In accordance with its general position, we are accustomed to describe as ectoderm the developing cell-plate, finally bent in the form of a hood, which occupies by far the greater part of the embryo, viz. the whole of the dorsal and lateral parts. The ventral and posterior parts are connected with the products of the lower cleavage sphere and partly envelop them.

"The most valuable part of the work (of Boveri), however, consists in the beautiful discovery that the separation of the germ-cells and soma which is theoretically required by Weismann is actually realised in the

case of *Ascaris*. After a very few cell-generations the primary sexual cell becomes separated. Its genealogy, the germ-track, marks a main stock with the whole, from which the primary somatic cells with their descendants are derived like so many side branches. Only the cells of the germ-track retain the original type of *Karyokinesis* and transmit it to the sexual cells of the growing worm: in the primary somatic cells, on the other hand, the nucleus disburdens itself of a certain part of its chromatin, and thenceforth follows a new and completely divergent type in all the mitoses of the body ('nuclear reduction')."

What is stated as to the two primary divisions of the egg of *Ascaris megalocephala* I have thus suggested independently, with modifications, in the human holophyte, and have carried back to an analogous extent the two forms of gametes. This shows really what the continuity of the germ-plasma involves.

The view I take of the ovarian and testicular teratoma is therefore this. It is a non-sex gamete which has retained from the primitive germ-cell the nucleus in part and the centrosome,[1] and thus the power of holophyte development normally lost by the gametes, and as a result we get "sample" potent determinants of the zygote, giving us the "sample" determinates found in the embryoma. These, as Wilms has shown, are normal in their development so far as they go. The loss of part of the nucleus and part of the centrosome takes away from the somatopleuric female gamete, normally formed, the power of holophytic development, and it can only regain this by restoring its nucleus and centrosome to full power of holophytic development by union with a dominant male gamete. If, however, the somatopleuric female gamete retains, after the segregation of the germ-cell, certain of the chromosomes, *i.e.* part of the nucleus and the centrosome, it will be in the position of being part of a zygote with the power of zygotic development, and can thus proceed on the lines of holophytic development to a limited extent, and thus form a teratoma (ovarian or testicular).

The primitive germ-cell and the primitive sperm-cell are really the same in essence. They are zygotic, are reduced by spermatogenesis and oogenesis to gametes, to prevent zygotic development, and this is restored by the crossing or fertilisation of a sex gamete (male in its origin) with another gamete, female and somatopleuric in its origin, and the zygote again formed. The stages are therefore: primitive germ-cell or sperm-cell *i.e.* zygotes (female or male); reduced zygote (gametes, male or female), crossing of gametes, as already given (p. 16); and zygote again. Fertilisation in the mammalia is thus a variation of gametes of unequal value, the

[1] What determines zygotic development is not exactly known, and thus the above statement is tentative.

same as when peas with contrasted characters are crossed, but the result is the tremendous one of a human fertilised ovum.

The description I have given of the formation of the primitive germ-cells and of their zygotic nature, their subsequent journey through the yolk-stalk and blastoderm till they "mobilise" on the Wolffian ridges, the possibility of their arrest *en route* and their modified reduction and partial zygotic development into embryomata, complete this view of the nature of teratomata, and give the pathologist a rational conception of their origin hitherto awanting. Thus dermoids or teratomata in the ovary or testis are derived from a somatopleuric gamete which has retained from its P.G.C. (really a zygote) what determines its development as a partial zygote into a teratoma. If the P.G.C. has been arrested on its way to the Wolffian ridges, and a similar imperfect reduction happens as in the former case, we get a teratoma at that site, outside the sexual gland.

I wish now to consider some other views as to the origin of *teratomata*.

(1) Shattock, in his valuable lecture, suggests a theory of epi-embryogenesis to explain the ovarian teratoma:—"This theory is that the ovarian teratoma results from the fertilisation of one of the primordial ova in the ovary so that the embryo gives rise to a second imperfect individual whose origin is therefore not synchronous with, but of later date than, itself. It is the formation of one embryo within another, and the name I venture to suggest for the theory is that of epi-embryogenesis. . . . It is well known that more than a single spermatozoon may perforate the investing membrane of the ovum. It becomes thus quite conceivable that a surplus spermatozoon may remain about the segmenting mass or morula, may become engaged between its component cells, and in this way be actually ready in the blastoderm to fertilise the primordial ova which are developed so soon after its lamination and the cleavage of the mesoblast which results in the formation of the body cavity" (*op. cit.*, p. 480). This is a most ingenious theory, but I need not criticise it, as Shattock himself points out its difficulties in the final paragraph of his suggestive paper. In the human male analogous teratomata occur, and a surplus spermatozoon in the morula coming in contact with a primary sperm-cell, *i.e.* of a similar nature, could not "cross" and form a teratoma of the testicle, even if one forms the hypothesis, as Shattock does, that "in the embryogenic sexual gland the proper reproductive cells are at first undifferentiated from one another. These cells become in the female the ova and in the male the spermatogenic cells of the testicle" (*op. cit.*, pp. 480 and 481). As a matter of fact, sperm-cells and germ-cells are such and nothing else at the beginning of development, and any idea of a hermaphrodite sexual primitive germ-cell in man is inconsistent with our present knowledge.

(2) Metaplasia, as Virchow put it, *i.e.* the origin of special tissues from the elements of other organs, does not give one much help.

Shattock's view may be classified with that of—

(3) Inclusio fœtus, and the latter is subject to the same criticism.

Okhubo, to whom I am indebted for information, considers—

(4) The Marchand-Bonnet theory as the most widely accepted. These look on a teratoma as a developed blastomere, *i.e.* as arising from an early division-product of the zygote, dislocated from its fellows and thus developing apart from its host. It may, indeed, be a fertilised polar body. It is said that this is supported by experimental evidence. It is akin to my view.

(5) Development of a germ-cell from some abnormal stimulus is urged by Fischel, and "Ribbert leitete Embryome aus einem keim, einer Zelle ab, die in der Hauptsache einer Eizelle gleichwertig war" (Okhubo, S. 542).

(6) Waldeyer, Wilms, and others would make embryomata arise parthenogenetically from the epithelial cells of the ovary, apart from fertilisation.

My own view is, as I have stated, that teratomata arise from a partially reduced primitive germ-cell or primitive sperm-cell, *i.e.* from the somatopleuric or non-sex variety of gamete. The primitive germ-cell I consider a zygote, according to the Owen-Weismann law; normally it loses its power of development to a holophyte by reduction to "gametes," but if this reduction is not complete, so that the somatopleuric gamete retains a power of zygotic development, we get a teratoma, a part of a holophyte. No other theory accounts for the teratoma being in the main only a definite part of an embryo and its never having genital determinates. It may be termed a "gametic theory" of the origin of teratomata, with the qualifications already stated as to its imperfectly reduced condition.

What happens when the primitive germ-cell is reduced to gametes?

I have stated that I believe the primitive germ-cell to be a zygote: spermatogenesis and oogenesis reduce it to gametes (sex and non-sex). Each gamete has half the number of chromosomes a somatic cell has, and thus Weismann holds that the maternal and paternal characteristics are equally divided. The objection to this deduction of a great observer is, that it does not square with the facts as observed in heredity, nor with the results of Mendel's experiments.

I do not attempt to explain how the dimorphic gametes are formed. I consider two forms necessary to give such a heterozygote as I conceive the human zygote to be, in view of the undoubted presence of potent (dominant) and non-potent (recessive) genital determinants in it. The analysis of the determinates in the genital unit seems to me to show that the genital unit-characters, dominant and recessive, are present in the same gamete. As I

shall show presently, the dominant and recessive sexual unit-characters separate out in certain cases of twins in black cattle.

The work already done in regard to the chromosomes is remarkable and most valuable, but we are yet in the dark as to whether they contain the unit-determinants concerned in heredity, or are directive in their division of the cytoplasm, and at present one must adopt a waiting policy.

The fact that M'Clung has found dimorphic sperm-cells in certain insects, and that there is in one of these an accessory chromosome, and that when this unites with an ovum, a female zygote is the result, is suggestive of a sex spermatozoon uniting with a non-sex ovum. No difference in ova has been yet noted in these cases, and there is thus as yet no evidence of dimorphic ova. Heape has not been able to substantiate Russo's work as to dimorphic ova.

Reduction and Division of P.G.C. to form Female Gametes.

Female P.G.C.
I F

Somatic somatopleure d'tants. D & R. } Is a part of the zygote;
Somatic d'tants for splanchnopleure and genital unit. D & R. } gives rise to II F & III F.

Non-Sex Ovum[1] *or Gamete.*
II F

−D & −R Somatic somatopleuric d'tants.

Sex Ovum[1] *or Gamete.*
III F

−D & −R Somatic d'tants for splanchnopleure and genital unit. D & R.

Female Zygote.
II M × III F
= IV F

D & R Somatic d'tants for somatopleure. } A part of this forms the P.G.C. mass and the P.G.Cs.
D & R Somatic d'tants for splanchnopleure and genital unit. }

Embryoma Ovarii.

Non-sex ovum *plus* part of nucleus and centrosome, *i.e.* d'tants for somatopleure *plus* part of centrosome.

F = Female. D = Dominant. P.G.C. = Primitive germ-cell.
M = Male. R = Recessive. P.S.C. = Primitive sperm-cell.
− = part of.

[1] Two varieties of ova have not yet been so clearly demonstrated as in the spermatozoon.

Reduction and Division of P.S.C. to form Male Gametes.

Male P.S.C.

I M

Somatic somatopleuric d'tants. D & R. } Is a part of the zygote: gives rise to II M & III M

Somatic d'tants for splanchnopleure and genital unit. D & R.

Non-Sex Male Gamete[1] *or Spermatozoon.*

II M

−D and −R Somatic d'tants for somatopleure.

Male Sex Gamete[2] *or Sex Spermatozoon.*

III M

−D and −R Somatic d'tants for splanchnopleure and D & R genital unit.

Male Zygote.

III M × II F

= IV M

D & R Somatic d'tants for somatopleure. } A part of this probably forms the P.S.C. mass and the P.S.Cs.

D & R Somatic d'tants for splanchnopleure and genital unit.

Embryoma Testis.

Non-sex spermatozoon *plus* part of centrosome, *i.e.* d'tants for somatopleure *plus* part of nucleus and centrosome.

F = Female. D = Dominant. P.G.C. = Primitive germ-cell.
M = Male. R = Recessive. P.S.C. = Primitive sperm-cell.
− = part of.

The reading of the above table is that the female zygote IV F, produced by a crossing of the gametes II M and III F, gives off the primitive germ-cell mass, and from this arise the primitive germ-cells, which are still

[1] Has $n-1$ chromosomes of M'Clung.

[2] Has n chromosomes, *i.e.* accessory chromosome which may be the sex chromosome and contain sex determinants.

zygotes, but become reduced to gametes of two kinds; and these by cross union with similarly formed male gametes give us again the zygote. In this way we get a variation in the constitution of the new holophyte.

The Evolution of the Differentiation of Sex.

Sex probably became differentiated finally by mutation, after prolonged variation experiments in zygotic reductions. The ground for this statement will come up presently when I mention the case of the free-martin. De Vries has shown that species may arise suddenly, *i.e.* by mutation or discontinuous variation, as in the case of *Oenothera Lamarckiana* and an old American species, of which wild forms found in Hilversum, Holland, when sown, bred true to the parents from which the seeds were taken and not to the parent-form *O. Lamarckiana*. Apparently two new species had originated from an old one in a state of nature (Lock, *op. cit.*, p. 135). I can conceive, however, of variation acting on the primitive germ-cells giving gametes not fertile, and that "mutation" in the differentiation of sex might be considered as a variation result as to gametes, which was successful, and that its ultimate suddenness—mutation—does not necessarily exclude prolonged variation trials. The differentiation of sex has been noted by Punnett to occur suddenly in the case of the pea. "Sometimes there may be seen a few plants [of sweet pea] in a row which, though to all appearances healthy and vigorous, set little or no seed. The reason is at once apparent when a flower is pulled to pieces. The anthers are sterile, only containing a little degenerate pollen, and the flower is consequently incapable of the normal self-fertilisation which obtains among these plants. Such few pods as are formed presumably owe their origin to insect agency, for the female parts of the flower are perfectly normal and readily set seed when pollinated from another plant. The F^1 hybrids so formed are all normal, showing that the fertile anther is dominant to the sterile. In the next generation (F^2) plants with sterile anthers reappear approximately in the proportion of 1 : 3 of the fertile, which is in accordance with expectation. This case of the sterile sweet pea is of great interest from its bearings on the problem of sex. Looked at from this standpoint, we have here a unisexual form, a female, which has suddenly arisen from the normal hermaphrodite form by functional suppression of the male organs. It suggests that, in cases where the sexes are separate, this condition may have suddenly arisen from the hermaphrodite one. The story of sex, however, is too long and too complex for us to enter upon in this connection" (*op. cit.*, pp. 16 and 17).[1]

[1] Weismann also noted this (*v.* Eimer, p. 211).

The view I take of this most interesting observation is that the sterile anthers and the potent female part are the result of the formation of gametes of unequal value but suitable for variation.

It would be of great value to know from a botanist what elements exactly are present in the degenerate anthers in such cases.

Origin of Unequal Gametic Value.

An important theoretical question now arises, viz.—What is the cause of the unequal value of the gametes? The answer seems to me to depend on the view one takes of the primitive germ-cell. The view I have taken of it is that it is a zygote. This follows from the Owen-Weismann law, and is of far-reaching importance. The germ-cells are formed and reduced, and we then get the gametes for variation, *i.e.* for cross-fertilisation. It is evident theoretically that if the ovary and testes of human adults contained not ova or spermatozoa respectively, but primitive germ- or sperm-cells, *i.e.* zygotes, the deposit of one of these in the uterus would without any immediate fertilisation lead to a female holophyte or male holophyte each nearly the exact replica of its parent. This would practically be budding. It is here the power of variation steps in. Gametes of unequal value are formed, and by the crossing of these, variation in the holophyte is brought about. The gametes are of unequal value, one sex gamete and one non-sex. The sex determinants are dominant and recessive, but are both in the one gamete: the second gamete contains no sex determinants. I thought first that the one gamete might possibly contain all the dominant, the other all the recessive determinants. This is apparently not the case, as it is of importance that no segregation of D and R sex determinants should take place normally when the gametes are formed. The non-potent or recessive determinates in the genital tract I consider merely evidence that the zygote is an impure dominant, and not that dominant and recessive elements are segregated in two gametes.

Castle has published a paper applying Mendelism ingeniously to the "Heredity of Sex." His views differ from mine, as he holds that "all animals and plants are potential hermaphrodites, for they contain the characters of both sexes" (p. 94), and that "on the hypothesis advanced the zygote must in all cases bear both the male and female characters." I regret that space does not permit my discussing his views, but refer observers to his original paper, and to the consideration of it by Cunningham and Darbishire. I may say, however, that in the evolution of the germ-cell and the zygote the human zygote has reached the stage of an impure dominant, but is not hermaphrodite. The zygote of a hermaphrodite plant such as

the sweet pea is really not an impure dominant, but a double dominant and double recessive, and the occasional sudden segregation of the germ-cells in it gives us the intrinsic sterile form Punnett describes.

The theory of male and female ova seems to me misleading and inconsistent with the Owen-Weismann law. The primitive germ-cell gives us sex and non-sex ova—not male and female ova; and the primitive sperm-cell gives in the same way sex and non-sex spermatozoa.

The primitive germ-cell mass can give rise to varieties of ova merely, and the primitive sperm-cell to varieties of spermatozoa: the element of the opposite sex combined with that of the characteristic sex present in each splanchnopleuric gamete is not sexually effective.

Determinants differ in their period of life in the ontogeny of an animal. Thus the determinants of the gubernaculum have apparently only a short life-history, a power of developing and acting only for a period of a few weeks, until the inguinal canal and scrotum are canalised for the descent of the testes. The round ligament determinants are fewer, but live longer. In phylogeny, determinants may have also a limited life in evolution, *i.e.* may exist only for certain periods—long periods in the race-history, but still with an end to their career. In this way we may explain the disappearance of the pronephros, pronephric duct, Wolffian bodies, and other organs. I throw this out, of course, only as part of a mere speculation and working theory. On this view the epoophoron in the adult is made up of the determinates of surviving but dying determinants.

I may now sum up my results as follows before concluding with some general remarks on Mendelism and its value to biology:—

Mendelian action does not differentiate sex, but acts when sex has differentiated.

The human male and female genital units contain definite unit-determinates characteristic of its own sex (potent) and the opposite sex (non-potent), as well as unit-determinates, potent and characteristic, but bearing evidence of segmental modification for special sexual purposes: this last class is best seen in the female external genitals (*v.* pp. 8–11).

These genital unit-determinates may be considered as having in their zygote unit-determinants some of which are dominant (potent) and some recessive (non-potent) (*v.* p. 13).

The human zygote is thus an impure dominant or heterozygote.

In the zygote, in its early division, there is set aside a portion of itself as the germ-cell mass, for the future primitive germ-cells, and these, formed without reduction, pass into the organism to the Wolffian ridges. This I term the Owen-Weismann law, or continuity of the germ-cells.

The Owen-Weismann law is one of the most far-reaching importance,

and if ignored we lose a great part of the scientific basis of development and variation in heredity. Its nature and bearings may be summed up thus:—

(1) In the zygote a primitive germ-cell mass is formed, and the remainder has the somatic determinants of the holophyte or adult which the zygote develops into.

(2) The P.G.C. mass then gives rise to the primitive germ-cells. These are zygotes, but probably differ from the somatic portion of their zygote in having some determinants the somatic portion does not possess.

(3) When the P.G.Cs. join the developed result of the somatic half of the zygote, we get a holophyte which contains certain determinates in its soma, but also, in its ova or spermatozoa derived from the P.G.Cs., determinants, certain of which differ from those in the somatic portion of the zygote, and thus, when this holophyte gives rise to new organisms, these latter may contain determinates such as the soma of the mother or father holophyte did not possess.

(4) When an organism has determinates not expressed in the determinates of its parents, their determinants have been in the parent gametes, secluded in them, and not expressed in the determinates of the parents. In this way we may explain recession, and if certain determinants are expressed only in a special number of gametes, we may get their effect delayed—so-called atavism.

(5) We see why the gametes contain the unit-determinants of the zygote they form. The gametes arise from the P.G.Cs., the P.G.C's. from the P.G.C. mass, and this as part of the zygote gives the gametes ultimately formed, their unit-characters.

The primary germ-cells (and sperm-cells) are thus really zygotes.

The power of developing to a holophyte in the primary germ-cell or sperm-cell is prevented by their reduction into dimorphic gametes—oogenesis and spermatogenesis.

This reduction of the primary germ- and sperm-cells produces in each case two varieties of gametes, sex and non-sex, with a divided centrosome and nucleus, and they are thus incapable in themselves of partial zygotic development.

The new zygote is produced by a crossing or variation of the gametes, by a sex-ovum × a non-sex spermatozoon giving a female zygote, or by a sex spermatozoon × a non-sex ovum giving a male zygote.

We may get the primary germ-cell so reduced that the non-sex ovum contains its share of the nucleus *plus* the centrosome or, put generally, what determines zygotic development; thus a somato-pleuric (non-sex) ovum may develop a teratoma in the ovary or testis, or indeed in many other parts of the body (*v.* p. 21).

Mutatis mutandis, we get in a similar way a testicular embryoma.

The non-sex spermatozoon contains, in the main, the somatopleuric determinants: the sex-ovum, the sexual and splanchnopleuric determinants. These crossed give the female zygote.

The non-sex ovum contains, in the main, the somatopleuric determinants, the sex spermatozoon, the sexual determinants, and splanchnopleuric ones. These crossed give a male zygote.

The human male holophyte has, in the main, primary sex and splanchnopleuric elements paternal: somatopleure and derivatives from the mother.

The human female holophyte has primary elements of sex and splanchnopleuric elements from the mother: somatopleure and derivatives from the father.

Fertilisation is thus gametic variation: the gametes are unequal in determinant value, one sex, the other non-sex. D and R somatic determinants may be present in both gametes, but the gametes are quantitatively of unequal value.

The result of gametic variation is to give us genital-unit dominant and recessive elements from different origins in the zygote, and finally in the genital-unit determinates.

The Owen-Weismann law applies to the formation of the germ-cell mass. The determinants of the P.G.C. are divided between the gametes, but D and R determinants of the G.U. tract are present in the same gamete, and there is therefore not any gametic segregation or purity of gametes in Mendel's sense.

The law of segregation does not act on the D and R sex determinants of the human zygote. They are normally inseparable and in one gamete.

The law of segregation, by a special action on the zygote, produces anomalous sex in certain animals, as I hope afterwards to show more fully.

Thus, in the condition known as the free-martin,[1] found not infrequently in the twins of black cattle, and hitherto considered an unsolved problem, this sterile animal is really an imperfect bull, as Spiegelberg showed, and the explanation of this anomaly, according to my views, is that the twins arose from one zygote, and that the potent G.U. determinants have segregrated for the potent twin, the recessive for the defective twin. Each gets a share of the testis, according to the Owen-Weismann law, after the primary division forming the twin condition took place. The potent twin is an extracted dominant: the free-martin an extracted recessive, *qua* their genital determinates.

In bees, probably, the three forms are queen (female), drone (male), and

[1] Occasionally the twins of black cattle are a normal bull-calf and an apparent female and sterile calf, the free-martin.

female-neuter. The current views seem to me very forced and improbable, and I am at present considering the question of Mendelism, or a modified Mendelian action, in regard to the origin of their various sexes.

My observations on these points are, however, not yet complete: but I may say that the present opinion that the drone, a perfect male, arises from an unfertilised egg, seems to me the most extraordinary ever held in embryology.

It may be that zygotic segregation is carried out in bees so that the dominant genital determinates are thrown into one primary division, the recessive into another, and thus a large number of holophytes produced with the recessive or non-potent sexual element—holophytes effective for work, the worker bees, but cut off from any power of propagation.

The Mendelian scheme may be divided by a transverse line immediately below F^1: the laws above act in normal sex, the law below (segregation) in anomalous sex, as in the free-martin and bees.

The differentiation of normal sex has been, in its final stage, in all probability a mutation.

The aim of gametic union is to get variation. This could not be got if the P.G.Cs. were allowed to become holophytes, but is attained by unequal gametic segregation and crossing of the gametes. Thus the production of normal sex, and the formation of a variation-holophyte, are guided by the same Mendelian mechanism as that acting on somatic unit-characters in plants—short, however, of the law of segregation, which would interfere with the formation of normal sex by producing abnormal forms of it. When the Mendelian mechanism goes on to F^2, we then get in black cattle pure extractives D and R.

Remarks on Mendelism in General.

Mendel's experiments on variation on the eating-pea are the most far-reaching that have ever been made in the study of evolution. Biologists have by this time a great record of such experiments, and these must now be verified, extended, and explained. Under these three heads there is much to be done, and we are hardly over the border of the land to be possessed.

Mendelism, as at present understood, in regard to pea-experiments, comprises two great facts and a theory. Dominance and Segregation are the facts, and the theory is that of Gametic Segregation. Of the facts, the Segregation seems to be undoubted, and is probably the best understood in fact, if not in mechanism; dominance is a term for a fact, but it is more

difficult to state what it actually involves, and still more difficult to explain its mechanism. The same criticism may be made as to the term recessive but I have already hinted that it may mean recession of a unit-character determinant into the gametes. The theory of gametic segregation, in explanation of Mendel's pea-experiments, I believe to be erroneous. I make these statements with great diffidence and at present under reserve; but only good can come from such reconsideration, and I am certain that what is wanted is first to make sure and certain our knowledge of the elements of Mendelism before applying it to the much more difficult higher problems in mammals. I defer, however, at present further criticism on this important point.

I began this inquiry as an attempt to ascertain if Mendelism acted in human development in the differentiation of the human genital tract. The question may now fairly be asked, How far have these processes been found to be guided by Mendel's laws? My answer in conclusion is that Mendelism is certainly the explanation in the problem before us—Mendelism acts when sex has become differentiated,—but that I have, with all diffidence, modified it. In the first place, I have not found the theory of gametic segregation satisfactory. It implies that characters on which Mendel's laws act are segregated in different gametes. The segregation of the D & R determinants in Mendel's pea-experiments seems to me to occur in what we may term the zygote, and the proportions of 1 : 2 : 1 are the result of zygotic segregation, not gametic segregation. I have found that probably in the human species the D & R unit determinants of the G.U. tract may be combined in one gamete.

Dominance is apparent, but is in a unique way; the potent genital elements are evident in the adult, but the recessive elements are also present, diminished in amount and simpler in structure. The D and R sex-characters are combined in one gamete.

The dominant and recessive sex-elements are an inseparable autonomous unit-character in the human species, but are segregated in the free-martin twins.

In anomalous sex, as in cattle and bees, segregation, I believe, acts on the zygotes and produces this condition.

The peculiarity of the recessive elements in the human G.U. tract is that they are modified and degenerated elements, definite in their anatomy, of the opposite sex. The same relation does not exist between the unit-characters in the peas on which Mendel experimented.

The essence of Mendelian action on differentiated human sex therefore seems to me: (1) the formation of P.G.C's. (impure dominants) and of unequal double gametes with autonomous unit-characters; (2) the dominance of one

unit-character normally, but the ultimate segregation of the pure dominant and of the pure recessive element *by zygotic segregation* in certain cases of anomalous sex only; (3) the transmission of unit-characters as an autonomous shuffling in the division of the zygote, not a dilution or blending; (4) the action of the hitherto neglected Owen-Weismann law, one result of which is that Dominance means the expression of a character in the holophyte; Recession, the temporary seclusion of a character in the gamete, but its reappearance in the succeeding generations by zygotic segregation or reduction.

It seems to me that the results brought forward point to the desirability of reconsidering Mendel's theory of gametic segregation.

Darwin and Wallace will always take the first place in the history of evolution, but Mendel I claim as its Napoleon, the greater than Napoleon, the unsurpassed master in the strategy and tactics of experimental variation. The practical closure of his work—a short eight years—by the unfortunate regulations of the Austrian Government as to the taxation of monasteries is probably the greatest misfortune Science has ever experienced.

I have to express my special obligations to Dr James Ritchie, the Superintendent of the Physicians' Laboratory, for much valuable criticism and advice.

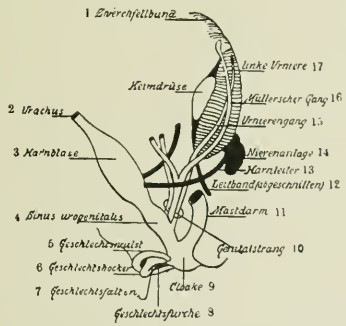


FIG. 1.—Diagram of urogenital system in type common to both sexes (Bonnet).

1. Diaphragmatic ligament.
2. Urachus.
3. Bladder.
4. Urinogenital sinus.
5. Prepuce.
6. Sexual eminence.
7. Sexual folds.
8. Sexual furrow.
9. Cloaca.
10. Genital cord.
11. Rectum.
12. Gubernaculum (cut).
13. Ureter.
14. Kidney rudiment.
15. Wolffian duct.
16. Muller's duct.
17. Left Wolffian body with testis.

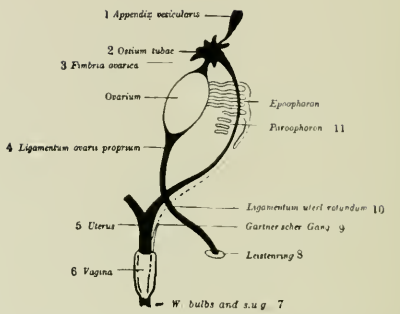


Fig. 2.—Diagram of female urogenital organs, showing ovary, tube, uterus, vagina, ovarium, and round ligaments, *Dominant*: epoophoron and paroophoron, *Recessive* and on male type (modified from Bonnet).

1. Hydatid.
2. Ostium tubæ.
3. Ovarian fimbria.
4. Ovarian ligament
8. Internal ring.

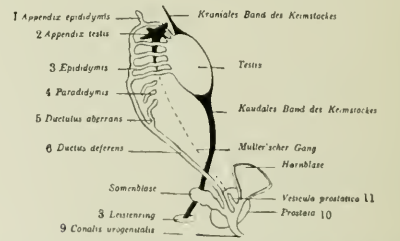
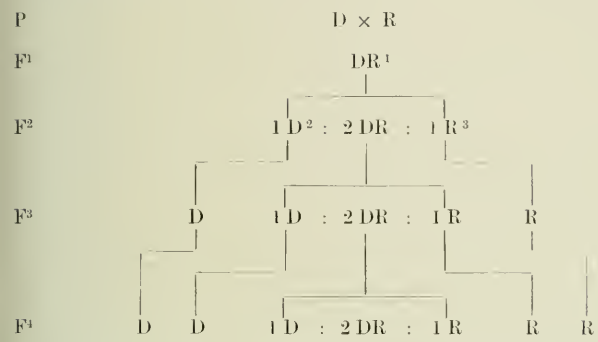


Fig. 3.—Diagram of male urogenital organs, showing testis, caudal ligament, gubernaculum, prostate, *Dominant*: appendix testis (Müller's duct), and prostatic utricle, *Recessive* and on female type (Bonnet).

6. Vas deferens.

In 2 and 3 the differentiated sexual organs are shown, arising from a hypothetical common type in fig. 1.

This diagram shows the place in Mendel's scheme of the human zygote and free-martin :—



DR = impure dominant ; D = pure dominant ; R = pure recessive ; P = parent ; F^1, F^2, first and second generation, etc.

[1] This is the equivalent in the Mendelian scheme for the human zygote.
[2] This is the equivalent for the potent twin bull.
[3] This is the equivalent for the free-martin, or sterile twin bull.
D × R in P represents the gametes.

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THE NATURE AND CAUSE OF THE PHYSIOLOGICAL DESCENT OF THE TESTES.

By D. Berry Hart, M.D., etc., *Lecturer on Midwifery, Surgeons' Hall, Edinburgh; Hon. Fellow, American Gynaecological Society; Carnegie Research Fellow.*[1]

PART I.—Descent in Marsupials.

"At this time of life the testis is connected in a very particular manner with the parietes of the abdomen, at that place where in adult bodies the spermatic vessels pass out, and likewise with the scrotum. This connection is by means of a substance which runs down from the lower end of the testis to the scrotum, and which at present I shall call the ligament, or gubernaculum testis, because it connects the testis with the scrotum and directs its course in its descent" (p. 6).

"It is plain from this description, that the cavity of the bag or of the elongation of the peritoneum which contains the testis in the scrotum, must at first communicate with the general cavity of the abdomen by an aperture at the inside of the groin" (p. 11).

"When the testis is descending and when it has even passed into the scrotum, it is still covered by the peritoneum exactly in the same manner as when it was in the abdomen" (p. 10).—John Hunter, "A Description of the Situation of the Testis in the Fœtus, with its Descent into the Scrotum," in *Observations on Certain Parts of the Animal Economy*, London, 1786.

"The parts in the female appropriated for the purpose of supplying the young are variously placed in different animals. In the horse, black cattle, sheep, and other graminivorous animals, their situation is between the hind legs, which is also the place allotted for the testicles of the male of this tribe; (and probably of all those in which they come out of the cavity of the belly) therefore in the hermaphrodite which has both these parts, the testicles are in some degree obliged to come down into the udder, which does not receive them so readily as the scrotum."—John Hunter, "Account of the Free Martin," in p. 45, *op. cit.*

In a previous communication I described a central canal in the ovary of the rat-kangaroo, apparently derived from the Müllerian duct. The question arose as to whether this was exceptional or normal in marsupials, and therefore, at Dr James Ritchie's suggestion, and through his kind services, I obtained from Professor Wilson of Sydney the pelvic ends of five kangaroo embryos in good preservation. Four of these were *Macropus ruficollis*; the fifth and largest, *Dasyurus viverrinus*. After suitable preparation, they were cut in transverse serial sections and stained with logwood and eosin. In this way about three thousand sections were

[1] This inquiry was carried out at the Laboratory of the Royal College of Physicians Edinburgh.

obtained. I now found that two were female and three male. This seemed at first a disappointment; but, on examination, the male embryos were found to be of great interest and novelty in many points, so that the results of their examination and of an extended investigation into the whole question form the subject of the present paper.

The results of the examination of the female specimens, so far as the development of the mammary pouch and round ligaments is concerned, will be found in another paper; but the conditions in the two sexes are intimately connected with one another, and must be read together to get a complete view of the subject. I found no central canal in the ovary.

In two of the male specimens (*Macropus ruficollis*) the Wolffian bodies were present with the developing testes on their inner and upper surfaces; the inguinal canal, gubernaculum, and cremaster had developed, and the scrotum was developing; in a third, more advanced, the Wolffian bodies had disappeared, the scrotum had developed and separated, and the testes were in the inguinal canal. One special point of value in the last specimen was that, although the testes were in the inguinal canal, its size was such that serial sections could be conveniently made. In the human male fœtus at this stage the specimens are too large for this being done.

I shall therefore take up—

I. The development of the scrotum, inguinal canal, and gubernaculum prior to the descent of the testes.

II. The condition of the scrotum and inguinal canal after the testes were in the inguinal canal.

III. The mechanism of descent of the testes as shown in the marsupial specimens.

IV. The descent of the testes in the human fœtus.

V. The phylogeny of descent of the testes and of the parts involved in it.

I. The Development of the Scrotum, Inguinal Canal, and Gubernaculum prior to the Descent of the Testes.

This was well shown in two specimens, but I describe the conditions in one only: the other corroborated these. The sections are first considered from above down, and begin a little above the suprapubic region.

Development of the Scrotum. Structure of the Abdominal Wall on Tr. Section.—The epidermis of the anterior abdominal wall in the suprapubic region has two ridges, one on each side of the middle line, the depression between the two lying opposite the linea alba. The deep epidermal cells are well marked. Behind it lies a layer of fibrous connective tissue, and

then comes a layer of denser connective tissue continuous on each side with lymph-sinuses to be presently described. This special layer has a homogeneous ground-substance interspersed with connective-tissue corpuscles and unstriped muscle. Next comes a layer of mesenchymatous tissue, and finally the peritoneum. Lower down on the abdominal surface the epidermic ridges are larger in section, and each has in it a circular space filled with

Fig. 1.—T.S. embryo, *Macropus ruficollis*; Wolffian bodies and testes in abdomen. (3.)
1, epidermal ridges; 2, lymph-sinuses; 3, pyramidalis muscle.

connective tissue of a myxomatous nature surrounded by deep epidermal cells. These are evidently sections of double solid cones in the epidermis. The sections of the cones below this lose their intermediate epidermal septum, and we get a larger hour-glass space. Then they lose their posterior epidermal wall, and the core has in it a double fan-shaped expansion of unstriped muscle derived from the inguinal fold, and broader in front (gubernacular fibres). There are thus formed in the suprapubic epidermic ridges double cones, complete above, but with the internal and

posterior boundary walls incomplete lower down. They are formed by the deep epidermal layer passing backwards and snaring in the connective tissue completely above, but less completely below, where the epidermal horns do not meet. These cones—best termed the scrotal cones—form the scrotum, into which the testicles ultimately pass. The fully formed scrotum is solid at first, but ultimately develops a cavity as the gubernaculum grows into it, as Klaatsch and Frankl and Katz figure in their specimens (figs. 1, 2, 3, and 4).

The marsupial scrotum has long been known to be prepenial, but the

Fig. 2.—T.S. *Macropus ruficollis* embryo. ($\frac{5}{4}$".)

1, phallus; 2, scrotal cone; 3, lymph-sinus. Note median partition disappearing.

reason has never been fully explained. All writers, except Klaatsch, consider it homologous and analogous to the ordinary scrotum in higher mammals. If so, however, it has not only appeared prematurely in phylogeny, but should not be prepenial and suprapubic. The account I have given of its development explains its nature. It is the analogue of the female mammary pouch, and, although this may seem paradoxical, its homologue too. It develops like the mammary pouch, but the deep epidermic layer passes backwards in crescentic form, the double fold containing little more than the deep epidermic cells, as the cavity of the pouch is to be formed, not by the desquamating epidermis as in the mamma, but from the enclosed connective tissue. This development of the scrotum in the suprapubic

region necessarily makes it prepenial, and the desquamation of the cells enclosed by the deep epidermic horns at a later stage renders the scrotum free and pendulous.

After making out the above developmental points in regard to the scrotum, I was glad to find it confirmed by Klaatsch's opinion, given in his paper. He there suggests that, from its position and relation to the gubernaculum—the analogue of the round ligaments—the scrotum must be the analogue of the mammary pouch. This question will be discussed, however, later on. I may mention here that it occurred to me that the adult scrotum

Fig. 3.—T.S., *Macropus ruficollis*. ($\frac{5}{1}$.)

1, gubernacular fibres in scrotum; 2, inter-sinus band displaced up and back; 3, horn of deep epidermis passing back; 4, solid scrotum formed from scrotal cones.

Fig. 4.—Gubernacular fibres in scrotum. ($\frac{7}{1}$)

1, epidermal ridge (3 in fig. 3).

might show a furrow in its mesial aspect as evidence of this development. The specimen Klaatsch figures does not show this, but it is well seen in the scrotum of *Acrobata pygmæa* figured by Katz. He describes this scrotum as heart-shaped, and states that he has seen it in young male specimens from Brazil, and in an adult specimen of the opossum (fig. 5).

In the male embryo where the testes were in the inguinal canal, the scrotum was separated from its epidermal bed and projected at right angles from the abdominal surface. It was still solid (fig. 15).

I may sum up this point here, then, by saying that the scrotum in marsupials is the analogue and homologue of the mammary pouch of the

female in every respect, but the full proof of the latter is only given in the section on Phylogeny (V.).

The Development of the Inguinal Canal and Gubernaculum.—The development of the inguinal canal and its contents, excluding the testes and epididymis in the male, is intimately bound up with that of the lymph-sinuses in the groin, and of the gubernaculum, acting in association with the muscular and fascial structures in the abdominal wall on which the inguinal fold, to be presently described, abuts. In both the specimens we are now considering, the testes were abdominal and developing on the Wolffian bodies, *i.e.* were abdominal and high up. This fact must be emphasised, as we shall see just now that the inguinal canal had developed

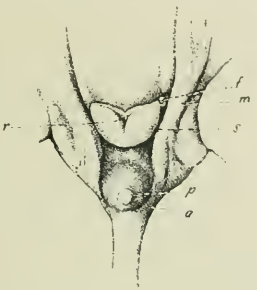


FIG. 5.—Scrotum in *Acrobata pygmæa*. (×.)
a., anus; *p.*, phallus; *s.*, scrotum; *r.*, groove between original cones. (Katz.)

while the testes were in the abdomen, so that, broadly speaking, the inguinal canal, contents and lining, tunica vaginalis, the gubernaculum (at this special stage), and cremaster owe their presence to a penetrating growth of gubernacular fibres, peritoneum, and cremaster along a definite lymphatic tract while the testes are abdominal, and thus not to any mechanical dilating process connected with the descent of the testicles. To understand this we must consider the structure of the abdominal wall. The anterior abdominal wall of the embryos, viewed on transverse section, has the following structure. First, on the surface, on each side of the middle line, is the epidermal layer with the two ridges already described, in which the scrotal cones are developed. Behind this, comes a thin layer of unstriped muscle, and then a thicker layer of connective tissue of a mesenchymatous nature, but with fibrous cells condensing it. Next this, lies a deeply stained band of connective tissue joining the inner ends of the lymph-sinuses; then

more mesenchymatous tissue; and lastly the pyramidales, recti, and transversales muscles, the external oblique and peritoneum, the last closely bound up with a fascia into which in the flanks the transversalis radiates. This fascia forms the transversalis fascia: the cord-covering known as the cremasteric fascia is derived from the tendon of the internal oblique. The fascial structures implicated in descent are well seen (figs. 1, 9, and 10).

I must now describe the lymph-sinuses as seen in the male. These, when examined in transverse section from above down, appear as an oblique chain of lymph-sinuses lined with nucleated endothelium, the connective tissue between the sinuses consisting of deeply staining round cells with a large nucleus; it thus stands out more markedly than the connective

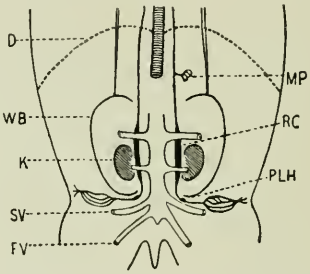


Fig. 6. — Lymphatic system at lower end of abdominal wall in 3 cm. long pig embryo (F. R. Sabin).

P.L.H., posterior lymph hearts; R.C, receptaculum chyli; K., kidney; W.B., Wolffian body; D., diaphragm; S.V., sciatic vein; F.V., femoral vein; M.P., ducts to mesenteric plexus.

tissue behind and in front of it. A very important landmark is, as we shall see, the band of connective tissue joining the inner ends of the sinuses. At the outer end of the lymph-sinuses lie an artery and vein We have thus in the mesenchymatous tissue of the anterior abdominal wall a ladder, as it were, of lymph-sinuses, of which we get transverse sections as we pass from below at the base of the scrotal cones obliquely up and out, showing on section the oblique inguinal chain I have just described. They spread up to form the superficial abdominal lymphatics, and in the specimens could be traced as far as the ribs. I traced them also in the sections down to a central group of sinuses lying behind the phallus, and this apparently poured by a deep channel into the Wolffian mesentery. The distribution of these lymph-sinuses is best understood by reference to papers by Gulland and Lewis, and especially to those of Dr Florence Sabin (figs. 1, 2, 3, 6, and 11).

In this valuable research Dr Sabin has shown that in the skin "the lymphatic system in the embryo pig begins as two blind ducts which bud off from the veins in the neck. At the very start the openings of these ducts into the veins are guarded by valves formed by the direction the epithelial bud takes as it buds off from the veins. In the ducts themselves there are no valves at first. From these two buds, and, later, from the two similar buds in the inguinal region, ducts grow towards the skin and widen out to form four sacs or lymph hearts, and from these sacs the lymphatics grow to the skin and cover its surface. At the same time there is a growth of ducts along the dorsal line, following the aorta to make a thoracic duct from which the lymphatics grow to the various organs" (p. 387, *op. cit.*).

In some of the illustrations Dr Sabin figures a chain of lymph-sinuses running in the line of the inguinal canal and also pouring deeply to the Wolffian mesentery. The nature of the lymph-sinuses in the groin which I have described in the kangaroo embryo is quite evident. They correspond to the lymph-sinuses in the pig, and are the posterior starting-points of lymphatic development for the skin of the anterior abdominal wall. Below, in my specimens, they unite in the tissue behind the phallus to form there a deeper single system of sinuses; these thus communicate with the superficial system Dr Sabin describes, and the latter can be traced in this specimen up as far as the ribs. Between the pubes and the deep system of sinuses lies connective tissue of a mesenchymatous nature (fig. 12).

Lewis has confirmed Dr Sabin's views as to the origin of the lymphatics, but found in the rabbit more than four centres of origin.

The outer edge of this sinus system, and the lymphatic tissue connected with it, corresponds with the outer edge of the pyramidalis on each side at this stage of the development of the inguinal canal; its upper limits are, at this stage, in the rib region, but may be higher; while, beneath, it ceases at the level where the inguinal fold passes in, and the muscles run from the front of the pubes to the hip bone; above this level the mesenchymatous tissue begins to be well developed. This tissue and the superficial abdominal lymphatic sinuses form the path along which the gubernaculum develops to form the inguinal canal and its contents (figs. 7 and 9).

The primitive inguinal canal, as I call it at this stage, runs from below up to reach the suprapubic scrotum, and thus transverse sections do not cut it in its full course. It will therefore be best now to describe the sections from below up, beginning at the point where the peritoneal dimple receives the inguinal fold, and passing up to the region of the scrotal cones. Before doing so, however, the nomenclature must be considered, as it is not in a satisfactory condition. The well-known term of Hunter, the guber-

naculum testis, is used in different senses, and embryologists have introduced new terms which human anatomists have not yet adopted. I use the terms "plica" or "fold" when the peritoneum is the naked-eye structure described: "gubernaculum" and "ligament" when the contained muscle or connective tissue is meant. In the stage at which the testes are still in the abdomen and attached to the Wolffian bodies, we have the following terms:—

(1) The *Wolffian mesentery* attaches the Wolffian body to the posterior abdominal wall, and its upper end is the diaphragmatic fold of the Wolffian body; its lower, the inguinal fold.

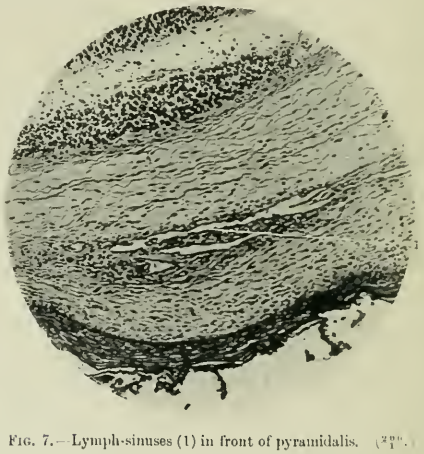


Fig. 7.—Lymph-sinuses (1) in front of pyramidalis. ($\frac{2}{1}$).

(2) The *testis* has an upper, cephalic, and a lower, caudal, fold or ligament. The former passes up to the diaphragmatic fold: the latter down to the inner and upper aspect of the genital ducts.

(3) The *inguinal fold* (plica inguinalis: gubernaculum before it penetrates the abdominal wall) is an important one. It is the caudal fold of the Wolffian-body mesentery, with an upper origin opposite the lower end of the caudal ligament of the testis, the width of the genital ducts thus separating them. Below, it ends at the inner abdominal ring. It is the peritoneal portion of the gubernaculum testis, and corresponds to the abdominal portion of the round ligament (figs. 8 and 9).

(4) *Gubernaculum testis.*—This is John Hunter's term, and is a valuable one. In using this term gubernaculum testis, or "rudder" of the testis, Hunter evidently did not mean to attach more than a "guiding influence" to it. It is often held to involve the idea of traction, but this is unfortunate. Hunter defines it as being the connection between the testis and bottom of the scrotum, and figures it in a six-months fœtus as extending down to that point. His line really, in the figure given by him, marks out the primitive inguinal canal. I shall use the term "developing guber-

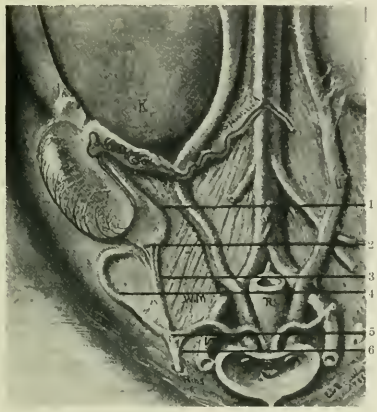


FIG. 8.—Pig embryo, 128 mm., injected and cleared (E. C. Hill) to show testis relations.

1, epididymis; 2, origin of caudal ligament; 3, caudal ligament; 4, ductus epididymis; 5, inguinal fold at origin; 6, inguinal fold.

naculum" as meaning the structure extending from the genital ducts to that point in the inguinal canal or scrotum it has developed to. This makes the caudal ligament a separate structure. It is thus the "developing gubernaculum" testis while the testis is in its original or early position. Its peritoneal portion is the inguinal fold. The term "developed gubernaculum" I shall use when the testis is in the inguinal canal or scrotum. Its upper end is in line with the caudal ligament of the testis and attaches it indirectly to the epididymis where the epididymis joins the vas deferens. The exact upper attachment of the gubernaculum is always really to the

epididymis, and *at the point where the globus minor and vas deferens meet* (Braman, Frankl) (fig. 8).

When the testis is passing into the scrotum, the gubernaculum begins to shrink, the involution of the gubernaculum. We should therefore speak of it at this time as the involuting gubernaculum. Its traces pass from epididymis to scrotal base. The gubernaculum, when it begins to penetrate the abdominal wall, is made up of a peritoneal roof like an inverted V, has unstriped muscle as its important constituent, and also connective tissue. It has at its lower end cremasteric striped muscle

Fig. 9.—T.S. embryo, *Macropus ruficollis*. ($\frac{5}{1}^{0}$.)

1, Wolffian body; 2, inguinal fold; 3, Müller's duct; 4, external oblique tendon; 5, inguinal wedge. Wedge beginning to penetrate into lymphatic tissue.

passing into it from below up, but this is a phylogenetic relic of the "conus inguinalis" of rodents and insectivora. At the caudal end of the developing and penetrating gubernaculum there grows with it, through the abdominal wall, the internal oblique and transversalis muscles, with the tendon of the external oblique in front of these. Thus the peritoneum of the inguinal fold, the unstriped muscle between its folds, and the internal oblique and transversalis muscles form a penetrating wedge which, as we shall see more clearly soon, invades a definite tract of lymphatic tissue. At a point in the anterior abdominal wall to the one side of the top of the pubes, and immediately above where the muscles in front of the pelvic wall pass out to the hip bone, the inguinal fold meets the abdominal wall,

without, however, penetrating it. It is then merely two lateral folds of peritoneum with connective tissue and unstriped muscle between them. A little above this we see the developing gubernaculum making its way through the wall. Here the peritoneum, fascia, the transversalis and internal oblique muscles, with the tendon of the external oblique in front, form a growing wedge which passes abruptly up and forward through the abdominal wall, towards the base of the scrotal cones. This penetrating wedge makes its way at first in the mesenchymatous tissue. Soon, in the succeeding sections, the internal peritoneal opening is lost, and we now get

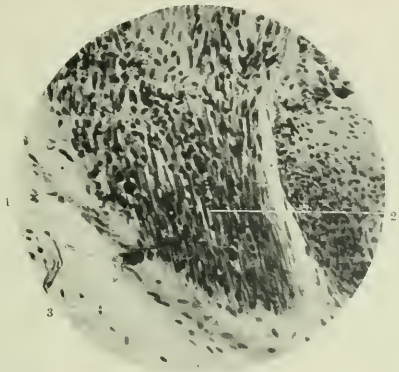


Fig. 10.—To show inguinal wedge ($\frac{2}{1}$").

1, tendon of external oblique; 2, wedge of transversalis and internal oblique muscles; 3, lymphatic tissue.

what Klaatsch terms the inguinal bursa—the inguinal canal with its coverings and main contents. We see the developing gubernaculum on section, with its partial peritoneal covering, and the part free from it and continuous with the connective tissue. Outside, comes the tunica vaginalis and the striped muscular and fascial coverings forming the walls of the bursa. These striped muscular coverings will ultimately form the cremaster. The inguinal wedge now passes on near the deep central lymphatic sinuses without invading them much, if at all, and next passes across the chain of superficial sinuses I have described and identified with those Dr Sabin has injected in the pig embryo. That the developing gubernaculum invades these sinuses, there is indubitable evidence. It has been already pointed

out that these superficial abdominal sinuses, higher up than this level, have their inner ends joined by a deeply stained band of connective tissue and unstriped muscle, the lymph-sinus band, thus forming with the sinuses a continuous crescent on transverse section. At this lower level, where the developing gubernaculum is penetrating the superficial sinuses, some of these lie to the outer side, while the joining band lies distinctly to its inner and posterior aspects. The developing gubernaculum thus actually traverses the sinuses. The gubernacular wedge now curves up to the open base

Fig. 11. –T.S., to show gubernaculum and cremaster. ($\frac{5}{1}$°.)

1, gubernaculum ; 2, cremaster with future inguinal canal between 1 and 2 ; 3, inter-sinus band displaced by gubernacular wedge ; 4, lymphatic sinus.

of the scrotal cones, the unstriped fibres alone radiating into the cones as already described (p. 246, and fig. 4). The tunica vaginalis and the cremasteric fibres are now absent (figs. 10, 11, 12, and 13).

The developing gubernaculum with the peritoneum and cremasteric fibres thus forms the inguinal canal with its tunica and cremaster by a growth of its wedge-shaped end in the line of lymphatic tissue and lymph-sinuses. The development of the sinuses at this stage is inside the outer edge of the pyramidalis, and it is then the gubernaculum, with the associated peritoneum and muscle, begins its growth, and its course seems to be influenced by this local lymphatic area.

Dr Sabin states: "In the posterior part of the body there are two centres for the radiation of the ducts—first, over the crest of the ilium for the ducts of the back and of the hip; and secondly, in the inguinal region, for the ducts that grow into the abdominal wall and down the leg. The ducts of the anterior and posterior system anastomose freely over the body."

It is thus at a certain stage of the development of the inguinal lymphatic system that the gubernaculum has its way shown, and this seems to me a new and valuable fact in embryology, viz. that of lymphatic

Fig. 12, as in fig. 11.—To show deep central lymphatic system. (5°.)

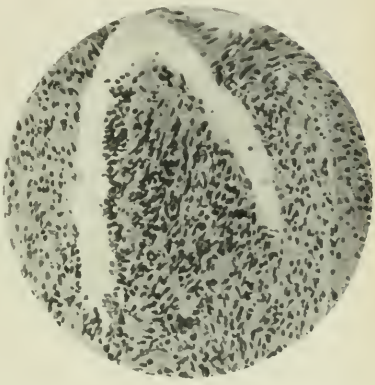


Fig. 13.—Gubernaculum in preformed inguinal canal. Note its basal attachment preventing its acting as a tractor. (2°°.)

distribution influencing the line of growth of other structures. So far, then, we see that in these embryos the growth of the developing gubernaculum has not influenced the position of the testes, as these are still high up in the abdominal cavity. It has formed the inguinal canal, the tunica vaginalis, and the cremasteric coverings with its fasciæ only; the cavities of the tunica and cremaster are merely potential, not as yet actual. The next point to consider is as to how these potential cavities are influenced by the change in position of the testes.

I have spoken of the inguinal wedge at the caudal end of the developing gubernaculum as growing in a lymphatic tissue and sinus track, and thus

forming the inguinal canal. It is made up of peritoneum, striped muscle (cremaster), and with unstriped muscle in the peritoneal fold. Which of these is the active one? The peritoneum is usually considered to lead the way, and to push the others before it, as it were. I think this wrong, and my reasons are as follows. The round ligament has only a little peritoneal invagination: the rest is penetrating unstriped muscular fibre. Then again one sees in the developing gubernaculum, in the appropriate sections, the unstriped muscle radiating into the scrotum ahead of both peritoneum and striped muscle. I consider the unstriped muscle the active penetrating tissue. Its occasional abnormal course in the human wall seems to show this. We thus get the perineal testicle in the male; and in the female its perineal penetration gives an anomalous and very rare perineal hernia in women, as in a case described by Smyly and one in my own practice. Here the hernia comes down below the lower end of the labium majus, and is best explained by supposing we have here an abnormal burrowing of the unstriped muscle of the round ligament analogous to the abnormal burrowing which would be followed by the testicle in the male.

Lockwood, in his valuable memoir, describes the gubernaculum in man as having insertions in the groin, scrotum, pubes, and root of penis. In the marsupials, the pubic fibres of man are represented specially; in the rodents and insectivora, the inguinal ones; and in man, the scrotal ones.

The question arises here as to the penetrating power of the gubernacular fibres. We know that the early division cells of the fertilised ovum in the mole have a phagocytic action, considered now to be of a biochemical nature. The action of the gubernaculum at any rate seems to me to be of the same nature; but this is a difficult point, and one that I suggest more as probable than demonstrate as actual.

II. The Condition of the Scrotum and Inguinal Canal, after the Testes were in the Inguinal Canal.

One embryo in perfect preservation was available for this stage. The scrotum had developed and separated so that it projected from the anterior wall, to which its base was attached; but it was still solid, with the gubernacular fibres radiating into each half. The inguinal canal on each side had undergone remarkable changes. It was markedly dilated, and contained the testis, gubernaculum, and epididymis. The inguinal wedge had thus differentiated and formed a large and roomy sac, the cremasteric sac, lined with peritoneum, for the testis. The gubernaculum and testis are thus caudal in the canal, with the epididymis cephalic in the sac and on a level with the testis. To understand these remarkable changes,

we must now study the serial sections, at first, from above down. No lymph-sinuses can be seen in the abdominal wall, as were noted in the earlier embryos. In the site of the inguinal canal, high up, and really cranial in its topography, there is first to be noted to the outer side a large lymphatic gland with some fat. Then appears, to the inner side, the inguinal canal, circular on section at this level, with a well-marked striped and unstriped muscular wall and a granular epithelial lining. As we pass down, the canal soon has its transverse diameter wider, and its lining of a

Fig. 14.—Inguinal canal with testis in left canal.

1, lymphatic gland; 2, inguinal canal; 3, scrotum; 4, testes. *Dasyurus viverrinus* embryo.

Fig. 15.—Inguinal canals with testes.

1, testes; 2, Muller's duct; 3, Wolffian duct; 4, gubernaculum; 5, scrotum with gubernacular fibres radiating in.

flattened endothelial type. The testis next can be seen lying apparently free in the canal,[1] but at a lower level in the sections it has the gubernaculum *plus* the caudal ligament attached to its caudal end, while at the caudal end of the canal there is special thickening of the cremaster, of its longitudinal fibres, below it. This is an important section and merits full description. The inguinal canals here lie side by side and are triangular in shape, the long base to the lower and outer side. The gubernaculum is continuous with the epididymis, attached at one part near the lower end of the testis, with the Müllerian and Wolffian ducts each at one level and

[1] Most probably the testes and sac walls were in contact originally, and floated out as the result of the necessary manipulation in mounting.

in a bulging thickening. The cephalic part of the epididymis is not shown but appears in the lower sections. The gubernaculum testis, at its lower insertion in the canal here, runs up a little on its walls, so that its caudal attachment between peritoneum and cremaster is really a concave, disc-like one, on section like an anchor (figs. 14, 15, and 16).

The septum between the two inguinal canals is well developed, with unstriped muscle in the lower half, fatty tissue in the upper (figs. 15 and 16).

Lower down in the sections a unique point is to be noted, viz. an opening

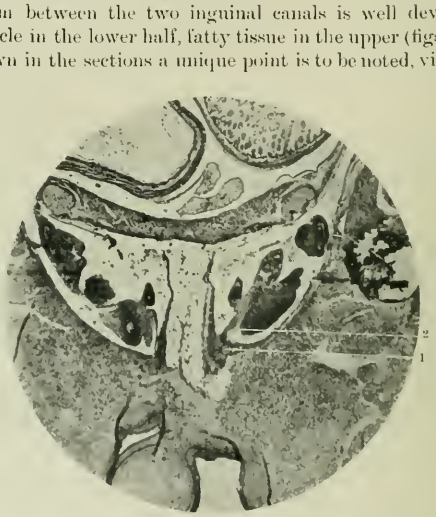


Fig. 16 as in fig. 15.—Shows union of cremaster and gubernaculum. ($\frac{2}{1}$°.)

1, cremaster; 2, gubernaculum.

in the upper and outer wall of the canal where the testis must have passed into the canal, or the canal developed up over the testis. Here a fold, the diaphragmatic fold, is continuous with the outer boundary of the peritoneal cavity. The edges of the aperture are thinned and pointed, and not in any way ragged, and give the impression of a gradual thinning and not of an artefact (fig. 17).

The *cremaster* is well developed. In the earlier embryos, where the primitive inguinal canal is being formed, it was noted that the developing gubernaculum had in front of it the internal oblique and transversales muscles, with the tendon of the external oblique in advance. In the present specimen the cremaster surrounds the dilated canal in its whole

periphery, with special thickenings at the cephalic and caudal ends. It arises as follows. A little to the outside of the top of the marsupial bone, the combined internal oblique and transversales muscles split into two lamellæ, the inner passing along the upper wall of the canal, the outer round the outer wall to meet its fellow and thus line the canal. The fibres pass up above their origin, as the pouch end of the inguinal canal is up in the abdominal wall considerably above the internal ring. Outside, the cremaster has a fibrous covering derived in its lower aspect from the fibrous tissue

G. 17.—Inguinal canal with opening above communicating with peritoneal cavity. Note thickening of cremaster at lower end. (4°.)

Fig. 18.—Shows caudal end of cremaster thickening (1). (2½°.)

outside the transversales. If now we compare the primitive inguinal canal with the developed one containing the testis, the following points are worthy of notice. When the primitive canal is being formed, the testes are high up in the abdomen, on the inner side of the Wolffian body, which is still large. From the caudal end of the epididymis the caudal ligament passes down to a point on the inner aspect of the genital ducts; and from a point in the genital duct wall opposite this the inguinal fold of the gubernaculum arises, passes to the internal abdominal ring, sends its growing fibres along the inguinal lymphatic track, to end in the scrotum and thus complete the gubernaculum (fig. 8).

When the testis is in the developed inguinal canal, the apparent

gubernaculum (developed gubernaculum) reaches from near the caudal pole of the testis to the base of the scrotum, but really represents, from above down, the caudal ligament of the testis and the gubernaculum. The testis has the mesepididymis with which the primary mesorchium has blended attaching it to the scrotal wall.[1]

A very important point is that the inguinal canal has increased markedly in size, that the testis lies in it loosely with room to spare. In the primitive inguinal canal and in the peritoneal cavity the developing

Fig. 19.—Testis (2) and primary mesorchium (3) with Wolffian body (4). The secondary mesorchium is formed by union of 3 and 4 when latter shrinks; 1, kidney. *Macropus ruficollis*. ($\frac{2}{1}\frac{0}{0}$.)

gubernaculum was an attached structure with only part of its periphery covered by peritoneum, and therefore unfitted to act as a tractor. The same holds good of the testis and gubernaculum when the testis is in the inguinal canal or scrotum. The testis has its secondary mesorchium, and the gubernaculum also has its attached aspect: the latter can thus not act as an actual tractor.

[1] There is thus a primitive mesorchium while the Wolffian bodies are still present; but when these atrophy, their peritoneal folds or mesentery—mesepididymis—blend with the mesorchium and thus form what we may call the "secondary or ordinary mesorchium" of the surgeon. Lockwood terms it the mesorchium, but the term secondary mesorchium recalls its strict origin.

III. The Mechanism of the Descent of the Testes as shown in the Present Specimens.

I wish now to explain how the descent of the testes has taken place in the marsupial embryos examined. Two of the specimens showed the testes in the abdomen and the primitive inguinal canal formed. In the third the testes were lying, each in the much-expanded inguinal canal. The scrotum was present but solid.

We have to consider the descent in three stages:—

(*a*) *The change in position of the testes so that they lie at the internal abdominal ring.*—I have no marsupial specimen to show this, but it has been described in the human and other fœtuses, and is not a difficult one to clear up, nor one on which much difference of opinion exists. When the Wolffian body in great part disappears, the testis naturally occupies its position, and thus sinks. The testis in the first two specimens lies to the inner and upper side of the Wolffian body, and thus guided by the inguinal fold comes necessarily to lie at or near the internal opening of the inguinal canal. No traction by the inguinal fold or by the rudimentary inguinal cones (*v.* V.) is necessary to explain this movement. Probably when actual intermediate stages are examined it may be found not to be a mere descent all the time; there may be a temporary stage of ascent, indeed, as in the human fœtus; but practically we may consider what I have described as probably correct.

(*b*) *Passage of the testis into the inguinal canal.*—This is a much more difficult stage to explain, but I hope to make it fairly clear. First I must notice that the third specimen shows an unusual condition of the developed inguinal canal, viz. that there is an opening in its upper wall to the inner side of the diaphragmatic fold. I have already stated that I do not think this opening has been artificially made in cutting the sections, as its edges taper; but until it is confirmed I do not insist on it as normal in the specimen. It makes no difference in the explanation of the change in position of the testes whether they pass by this opening or *viâ* the processus vaginalis. The important point is the enlarged condition of the inguinal canal. It is relatively much larger than the testis in it. At its highest and caudal point, and outside it, as I have shown, a lymphatic gland lies, and this and the fat round it, is the natural result of its development in a lymphatic tract; the increase in size has been due to growth.

Now, the reason the testis lies in the inguinal canal is not that it has been drawn into it, but that the inguinal canal has grown so large as to surround it; that is, the inguinal canal has, as it were, ascended to the testis and thus become its temporary home. It is thus not so much an actual descent of

the testis into the inguinal canal at this stage, as an *ascent* of the inguinal canal. All the specimens of marsupials at this stage figured by Katz and Klaatsch show, as my one does, the relatively great enlargement of the canal to the testis. In my specimen the testis now lies in the canal uncovered by peritoneum, but with germ epithelium in single layer as its outer envelope. Above, it is suspended by the representative of the diaphragmatic ligament; below, it has the developing gubernaculum attaching it to the lower end of the inguinal canal; and it has a mesentery, the blended mesorchium and mesepididymis, *i.e.* the secondary mesorchium. We have an analogous process in the stages of the relations of the female pelvic organs to the brim of the true pelvis. In the fœtus the uterus and bladder lie above the level of the pelvic brim; afterwards, in the adult female, they lie below its level. They have not descended or been drawn down, but in the development of the bony pelvis the brim of the true pelvis grows above them.

The same is seen in regard to the spinal cord. In the early embryo the spinal cord is seen in the lower end of the spinal canal; afterwards its lower end is much higher, a change due to disproportionate growth.

It will be seen that I have not given prominence to traction of the gubernaculum as an important cause of descent. I do not because it is unnecessary and unlikely. The gubernaculum in these specimens has only unstriped muscle; it has an attachment to the wall of the canal nearly in all its length, is not attached directly to the testis, and is not a cord, free at its periphery. It is thus not likely to be muscularly active in a dynamic or change-of-position sense. One cannot, of course, deny that it is not active to a certain extent, but its activity has not been proved and has been exaggerated.

(*c*) *Passage of the testis into the scrotum.*—In my specimen the testis was in the inguinal canal and the scrotum was solid. The scrotum was in course of being canalised, however, as the unstriped muscle of the gubernaculum was radiating into it, and peritoneum and cremaster were acting with it. The solid scrotum would thus soon have its cavity lined with peritoneum, and the testis in it.

How would the testis get into the scrotum? No doubt, as the scrotum became invaded by the growing gubernaculum, the testis would necessarily lie lower and enter it, a change permitted by its slack mesentery—would be carried into it with the involuting gubernaculum. Here again muscular action of the unstriped muscle of the gubernaculum may be invoked as the gubernaculum is shrinking. But it does not seem to me to be an important factor. The whole descent seems to me to take place by a series of growth processes. The second stage is best explained as an unequal growth

phenomenon; the third as due to gubernacular shrinking: but here those who believe in muscular gubernacular action may dissent. The disappearance of the Wolffian bodies, the directing action of the gubernaculum —the rudder more than the tractor—the disproportionate and marked growth of the inguinal canal, and the final involution of the gubernaculum: the play to the testis afforded by the diaphragmatic fold and the secondary mesorchium, its mesentery,—all these acting in series or in conjunction seem to me to account for the altered position of the testes.

In the marsupial it is, at its last stage, an ascent into the scrotal pouch, although on the whole it is a descent from its primitive position.

(*To be continued.*)

THE NATURE AND CAUSE OF THE PHYSIOLOGICAL DESCENT OF THE TESTES.

By D. Berry Hart, M.D., etc., *Lecturer on Midwifery, Surgeons' Hall, Edinburgh; Hon. Fellow, American Gynæcological Society; Carnegie Research Fellow.*

PART II.—Descent in Man.

IV. The Descent of the Testes in the Human Fœtus.

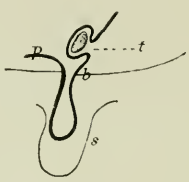
We are not yet in a position to explain descent thoroughly, but with a distinct approach to this. The first naked-eye and comparative work was done after Haller by John Hunter in his well-known paper published in 1786. Since that time, papers on the subject have been sparse in Great Britain, with the exception of those by Cooper (1830), Cleland (1856), Owen (1868), and Lockwood (1888). Thus in the literature summarised by Frankl in his paper in 1900, 121 references are given, but of these only three are British (Cooper, Owen, Cleland): and Lockwood, the most recent, is not quoted.

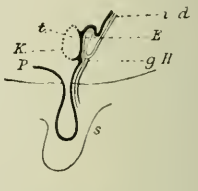
On the other hand, research has been abundant in Germany, less so in France, and important papers have been written by Bramann (1884), Frankl (1895–1900), Katz (1882), Klaatsch (1890), Nagel (1891), Weil (1884), Weber (1886), and by others.

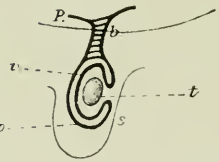
While one recognises in Hunter's paper *leonem ex ungue*, a large amount of comparative and microscopic work has been done abroad since his work, and very little if any has crept into our text-books and teaching. The reasons for this are that in the first place the idea that the abdominal wall was unbroken until, at the earliest, the 3rd month, and that at or about the 7th month the testes were drawn into the inguinal canal and scrotum by the gubernaculum, deriving their coverings during this progress, was held by many as a sufficiently exact account of the matter, although in several of our text-books the description of a preformed canal is mentioned so far as its peritoneal and even its muscular elements are concerned.

Then, again, an evident inaccuracy is present in all British and American text-books and most foreign ones, viz., the description of the testes as lying at first extraperitoneally in the abdomen and passing down into the scrotum extraperitoneally, either by muscular traction purely, or by the aid of mutual unequal growth of inguinal canal and gubernaculum, so that after the obliteration of the processus vaginalis we find a peritoneal covering to the testes (tunica serosa) and a peritoneal lining to the scrotum (tunica vaginalis). This mechanism is described in order to

give a peritoneal covering to the testis. I need not criticise these statements in detail, but may shortly say that: (1) The testes in the abdomen of the fœtus are not covered by peritoneum, but by germ-epithelium. (2) The testes are not extra-peritoneal in the abdomen after the Wolffian bodies have involuted, but have a distinct mesentery, in the main developed from the diminished Wolffian structures. (3) In the scrotum the testes are not covered by peritoneum. If they were, the peritoneum would strip off as it does from a tumour such as the epoophoritic (par-ovarian) developing in the broad ligament. (4) The testes in the scrotum are really covered with involuting germ epithelium as the ovary is (Frankl, Hoffmann). (5) However the human testes get into the scrotum, their route is *viâ* the processus vaginalis, into the tunica vaginalis, and then the processus becomes obliterated. John Hunter says this distinctly. I came to this conclusion during the study of







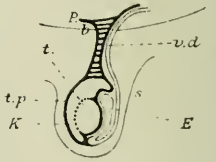


Fig. 20.—To show usual View of Descent and its Errors. (Frankl.)

t, testes; *P*, peritoneum; *b*, proc. vag. open; *S*, scrotum. The lower *b* shows obliterated proc. vag.

Fig. 21 —To show more exact View of Descent. (Frankl.)

v.d., vas. def.; *t*, testes covered with germ-epithelium; *E*, epididymis; *P*, peritoneum; *s*, scrotum.

my specimens, and was beginning to verify some points, but found it unnecessary to do so, on noting in the course of reading, that Frankl in 1895 showed clearly that "the testis has a peritoneal envelope (the tunica vaginalis), but not a peritoneal covering."

He points out, too, that Hoffmann (in the Quain-Hoffmann *Anatomie*, Erlangen, 1870) drew attention to this fact and showed the similarity of the testicular outer covering to the ovarian one.

Frankl's paper is of great interest. He shows that the testis, like the ovary, is not extraperitoneal, but covered by germ-epithelium. The fœtal testis in the scrotum is covered by low columnar epithelium—a contrast to the squamous endothelium of the adjacent parietal layer. He shows that the descent of the testes must occur through the processus vaginalis, and that then only the epididymis and inner wall of the scrotum are covered by peritoneum; the testes' outer covering is,

as already said, involuting germ epithelium. There is, indeed, an evident naked-eye boundary between testes and epididymis, corresponding to the well-known white line of Farre in the ovary. This makes the explanation of the descent very much easier.

We may now consider the question of how the testes descend in the human embryo. I base this account on my own specimens and on the facts given by Bramann, Weil, Eberth, Lockwood, Klaatsch, and Frankl. The papers of these observers are of the greatest value. In Wiedersheim's work the description, so far as it goes, is excellent and suggestive.

We may consider descent of the testes in man under the following heads:—

(*a*) The development of the testes in relation to the Wolffian bodies in the early embryo (about 4th week).
(*b*) The development of the preformed inguinal canal.
(*c*) The abdominal changes in position of the testes.
(*d*) The passage of the testes into the inguinal canal and scrotum.

(*a*) *The Development of the Testes in relation to the Wolffian Bodies.*

I need not go into detail on this point, but only mention facts relevant to the inquiry. Details of this early development are well given by Lockwood and in all text-books of embryology. The testes develop on the inner aspect of the Wolffian bodies, have a short mesorchium, and are recognisable as such to the naked eye by the 5th week. When the Wolffian bodies atrophy, usually about the 2nd month, this primary mesorchium of the testes is amplified by the Wolffian mesentery, and we thus get a secondary mesorchium. At this time (2nd to 3rd month) the testes lie in the abdominal cavity.

(*b*) *The Development of the Preformed Inguinal Canal.*

The material for determining this point is not great in the human male fœtus, but we have microscopic (serial sections in the main, by Weil, Klaatsch, and Frankl) as well as serial sections of two human female embryos (5th and 6th to 7th week) in my possession. If we summarise these as to sex and age, they are as follows:—

Male.—In a 14·5 mm. embryo (Frankl, measurement from head to breech) approximately 25 to 28 days, the caudal end of the Wolffian body and that of the Wolffian duct are placed at the abdominal wall: no inguinal fold, *i.e.* gubernaculum, is present.

In a 16 mm. embryo (28 days) the same conditions are present.

In a 28·5 mm. embryo (5th to 6th week) we have a marked change

(fig. 22). There is not only an inguinal fold but a beginning processus vaginalis. The inguinal fold has begun to penetrate, and a peritoneal dimple has formed. The transverse and internal oblique muscles are distinctly seen, but are, as yet, not beginning to penetrate, with the peritoneum and gubernaculum, as a wedge, through the abdominal wall. Into the base of the inguinal fold a few striated muscle fibres have radiated. The aponeurosis of the external oblique is also shown unbroken.

In a 4 cm. and 4·8 cm. embryo (3rd month) the peritoneal dimple was no deeper.

In an 8 cm. embryo (3rd month), Frankl figures the gubernaculum passing through the abdominal wall and presenting in the main the appear-

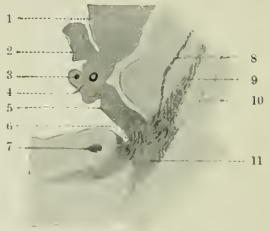


Fig. 22.—Trans. section through the body-wall, proc vaginalis, inguinal fold, and sexual gland of a male embryo, 28·5 mm. head-breech diameter. The mass of cells at 11 is traversed by muscular fibres.

1, Wolffian body; 2, testis; 3, duct of Müller; 4, Wolffian duct; 5, inguinal fold; 6, p.v. peritonei; 7, m.r. abdominis; 8, m. trans. abd.; 9, m. obl. uterus; 10, aponeurosis m. obl. extern.; 11, mass of cells. (Frankl and Eberth.)

ance I found in the *Macropus ruficollis* specimens (figs. 11 to 13). He divides the developing gubernaculum into three portions: an abdominal portion, a vaginal portion (in the peritoneal dimple), and an infravaginal portion below the level of the peritoneal dimple. It is into the last only that striated muscle radiates from below, the analogue of the conus inguinalis (*cf.* V., section on Phylogeny), and forms really what has been described as the ascending fibres of the cremaster.[1]

Klaatsch, in an 8 cm. embryo, figures these ascending fibres as well marked, and indeed as forming by an inversion of the gubernaculum into the peritoneal cavity a structure quite comparable with the conus inguinalis of rodents; and in fact in the 17 cm. embryo he figures the processus vaginalis as obliterated (shown in 8 cm., 11 cm., 15 cm., and

[1] This division of the gubernaculum comes up specially under the changes at the 7th month.

17 cm. (4th month) fœtuses). He would thus make the processus vaginalis be present as an inversion of this conus in the 17 cm. embryo. Frankl criticises this, and indeed it is evident that the peritoneal dimple or fossette is formed in 25 mm. embryos by, or along with, the passage of the gubernaculum through the abdominal wall.

As the sections of the Frankl 8 cm. embryo are followed down, we see how the processus is formed by the penetration of the double crescentic peritoneal folds, and finally at the lowest sections we come on the end of the developing gubernaculum, uncovered by peritoneum, and with the cremaster on all its aspects but the lowest. At or about this time (10 cm. embryo) the gubernaculum increases in size, mainly by growth of its connective tissue elements, and at this period, too, the external abdominal ring has formed.

In the 12 cm. embryo (4th month) the gubernaculum is deeper and the testis is at the internal abdominal ring.

In the 19 cm. embryo (5th month) the gubernaculum thickens and lengthens, and the testis rises a little from the internal ring—a real ascensus.

In the 23 cm. embryo (5th month) the processus vaginalis is deeper, and in the neighbourhood of the pars vaginalis of the gubernaculum there is striated muscle, and more of it in the infravaginal portion. This thickening of the gubernaculum may dilate the processus vaginalis, but probably there is a combined growth of the two.

At the end of the 5th month and beginning of the 6th, the aponeurosis of the external oblique and the cremaster fascia are everted along with the gubernaculum, which is now at the entrance to the scrotum. The gubernaculum is shorter, and striated muscle fibres (vertical and circular) are present in the infravaginal portion.

It must be noted that the ages of the embryos given are based on measurements, are difficult to give exactly, and are therefore only approximate.

There is thus complete evidence that in the human embryo, prior to the passage of the testes through the abdominal wall, there is a preformed inguinal canal, due to a passage of the peritoneum, gubernaculum, and transverse and oblique muscles, to the outer side of the rectus, forwards and inwards towards the scrotum.—It happens as in the marsupial embryo, with the difference that the gubernaculum contains scrotal, not abdominal unstriped fibres, and that the marsupial scrotum is suprapubic and not perineal as in man. None of Frankl's or Klaatsch's drawings show lymphatics, but this is probably merely an omission. I found them in relation to the developing round ligament, as I shall explain in a subsequent paper.

(c) The Abdominal Changes in Position of the Testes.

These have been given with great accuracy and clearness, so far as dissection can go, by Bramann, who examined forty specimens, and his results may be briefly summarised as follows:—

In a specimen *at the end of the 2nd or beginning of the 3rd month*, the testes 3 mm. × 1·3 mm. were about 1 mm. from the internal abdominal ring. Behind them lay the epididymis: the vas deferens ran in a horizontal direction to the bladder. From the point where the vas deferens issues from the epididymis, or, as Frankl puts it, at the junction of the globus minor and vas, the gubernaculum, 1 mm. long and ·5 mm. broad, passed to the internal ring, where there was a shallow peritoneal depression—the beginning of the processus vaginalis.

At the end of the 3rd month or beginning of the 4th, the testes lay lower and at the region of the internal abdominal ring. The testes were 4 mm. × 2 mm. in a 14 to 15 weeks' embryo, and close on the internal ring, with an inguinal fold ½ mm. long. The mesorchium was longer, and allowed mobility to the testis.

After this, the testes ascend somewhat, owing to the increase in length and thickness of the developing gubernaculum—its length and breadth at this period (13th to 16th week) being about 1 to 3 mm. by ½ to 1 mm. (average in seven specimens).

At the end of the 4th or beginning of the 5th month, the testes are larger (5½ mm. × 3½ mm.), the mesorchium is longer, and the upper portion of the epididymis has a mesepididymis (mesorchiagogos of Seiler). The gubernaculum measures 3 to 2 mm. in length. By dissection, from without, in the region of the external abdominal ring, and removal of skin, superficial fascia and aponeurosis of external oblique, one can see white fibres issuing from the external ring, and these pass to the external oblique aponeurosis.

Up to the *end of the 6th month* the gubernaculum seems to have attained its highest development, its length being from 3 to 8 mm., and its breadth, a little below the testes, 2 to 4 mm. The processus vaginalis is about 3 to 3½ mm. deep, and its entrance admits a fine sound.

At the *beginning of the 7th month* the real descensus begins. The testes, which were 5 to 8 mm. from the internal ring, now approach it, and the inguinal fold is shorter, the processus vaginalis deeper, so that a sound can be passed to the aponeurosis of the external oblique. The testes, as the age of the fœtus increases, still descend, and now pass to near the internal ring, and the processus vaginalis now projects from the external ring, covered by the external aponeurosis, a hollow cylindrical structure 6 mm. × 4 mm.

If the aponeurosis and peritoneum be incised we now come on the peritoneal sac, and can see, on the posterior wall, the gubernaculum about 12 mm. long, projecting into the sac-lumen for about $1\frac{1}{2}$ mm. without a mesentery, and reaching from the epididymis (where the globus minor meets the vas deferens, according to Braumann and Frankl) to the base of the inguinal canal.

In the $7\frac{1}{2}$ *month* the testes are now in the inguinal canal, the gubernaculum shorter; and when they pass the external ring, the peritoneal sac is covered by the unpenetrated aponeurosis of the external oblique, and the fibres of the internal oblique and transversalis. The lower end of the peritoneal sac is attached to the fascia superficialis, and not united to it by

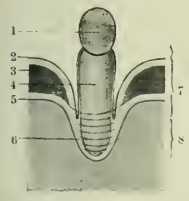


Fig. 23.—Position of testes to ligt. inguinal and proc. vaginalis in a 7th month fœtus.

1, testis; 2, peritoneum; 3, muscles; 4, ligt. ing. (gubernaculum); 5, ext. oblig.; 6, cremaster; 7, vaginal part of G.; 8, infravaginal part of G. (Frankl and Eberth.)

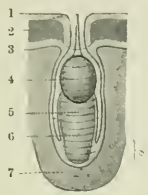


Fig. 24.—Deepening of proc. vag and approach to base of scrotum; 8th month fœtus.

1, peritoneum; 2, muscles; 3, external oblique; 4, testis; 5, gubernaculum; 6, cremaster; 7, scrotum; 8, vaginal portion of G. (Frankl and Eberth.)

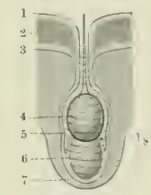


Fig. 25.—Shortening of vaginal part of gubernaculum in 8th month fœtus.

1, peritoneum; 2, muscles; 3, abd. ext.; 4, testis; 5, cremaster; 6, gubernaculum; 7, scrotum; 8, vaginal portion of G. (Frankl and Eberth.)

a rudiment of the gubernaculum. The fibres of the gubernaculum blend with the tissue of the processus. This is also what I have found in the marsupial embryo when the testis is in the inguinal canal. In fact the gubernaculum then spreads out as a thin layer between peritoneum and cremaster (fig. 16). The testes at last pass into the scrotum.

The changes beginning about this last stage have been well worked out by Frankl and Eberth. I have already spoken of the division of the gubernaculum into three parts by Frankl, and must now consider it according to his description in the 7th month fœtus. He gives three useful diagrams on this point.

In the first (fig. 23) the right testis is at the internal ring, and we see the abdominal part, vaginal part, and infravaginal part of the gubernaculum. The testis and gubernaculum show marks of contact with the small intestine.

On the left side the testis was much deeper, the lowest third of the gubernaculum being in the processus vaginalis.

In a third specimen at the 7th month, the testis has passed the inguinal canal, is partly in the scrotum, the processus vaginalis has begun to involute, and both the vaginal and infravaginal portions of the gubernaculum are shorter (figs. 24 and 25). Frankl's diagrams give the descent somewhat earlier than other observers.

Eberth gives an excellent figure of the relations at this time. Similar conditions may be found at the 8th month and in the newly born (fig. 27).

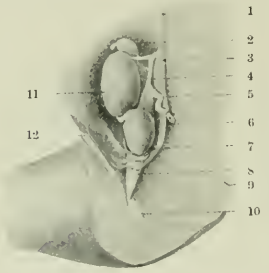


Fig. 26.—Male fœtus (11 cm. h.-b.), sagittal mesial section. ($1\frac{1}{2}$.)

1, peritoneum; 2, epididymis; 3, mesepididymis; 4, blood-vessels in mesepididymis; 5, ductus deferens; 6, inguinal fold; 7, entrance to proc. vaginalis; 8, inguinal ligament; 9, os coccygis; 10, symphysis; 11, testis; 12, body-wall. (Eberth.)

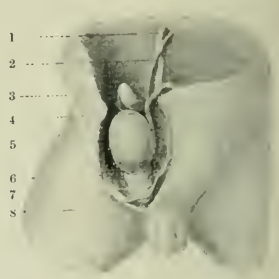


Fig. 27.—Male fœtus before birth. (1.)

1, ureter; 2, mesorchium; 3, epididymis; 4, d. deferens; 5, testis; 6, p. v. peritonei; 7, pars vaginalis, lig.-genito-ing.; 8, pars infravag. lig.-genito-ing. (Eberth.)

Increased growth of the processus vaginalis and shortening of the involuting gubernaculum, are the conspicuous features in the 7th to 8th month.

(d) The Passage of the Testes into the Inguinal Canal and Scrotum.

It may now be asked what are the causes of descent of the human testicle, and the approximate explanation is as follows:—

The disappearance in great part of the Wolffian body, and the guidance as a rudder, but not as a tractor, of the inguinal fold (gubernaculum at this stage), determine the position of the testes near the internal abdominal ring at or about the 3rd month (fig. 26).

The subsequent hypertrophy of the developing gubernaculum and its appearance in the peritoneal cavity as a thickened projection analogous to the conus inguinalis, if we follow Klaatsch's specimens of this period, cause a temporary ascent of the testicle. The hypertrophy with increased pro-

jection into the peritoneal cavity is a fact, whatever view as to its analogy to the conus in rodents we adopt, and has the result of causing the testis to lie higher. It may also have a dilating effect on the processus vaginalis: but as I have already said, there is more probably a combined growth of gubernaculum and processus.

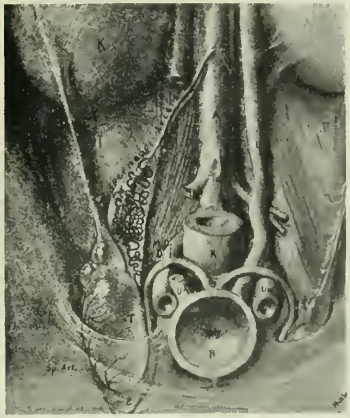


Fig. 28.— A transparent preparation of the right testis of an embryo pig, 210 mm. in length × 6.

Left testis nearly in inguinal canal; right testis, T, just entered; K, right kidney; A, dorsal aorta; E, epididymus; U, ureter; R, rectum; M.D., W.D., Müllerian and Wolffian ducts; U.A., umbilical artery. (Eben. C. Hill.)

The next stage (6th month to 8th month) is probably an increase in the capacity and length of the processus vaginalis, so that it expands and grows up, as it were, over the testis, enclosing it in the inguinal canal (fig. 28).

Owen has suggestive remarks on the presence of the more or less complete ovarian peritoneal capsule of the ovaries found in many mammals. "In the white bear (*Ursus maritimus*) the ovaries are completely enclosed in a reflected capsule of the peritoneal membrane, *like the testes in the tunica vaginalis*: a small opening, however, leads into the ovarian capsule at the part next the horn of the uterus" (*op. cit.*, § 99). This is an interesting comparison, as the ovarian capsule probably grows up round the ovary as I have described the inguinal canal enclosing the testis.

In the meantime the unstriped muscle gubernacular fibres with the striped muscle at its apex, and the peritoneum are developing into the solid scrotum, thus forming a cavity in it, lined with peritoneum. At this stage a

shrinking of the gubernacular fibres takes place, and this is one factor (with probably some play allowed to the testis by the secondary mesorchium or mesepididymis of Frankl) in determining its ultimate position in the scrotum.

It will be seen, therefore, that in explaining the passage of the testis into the inguinal canal, a growth and development of the canal and of the gubernaculum, and not an actual descent of the testis, is considered the great factor. This is well demonstrated in the marsupial specimens, as well as in those of Klaatsch and Frankl.

I have said little of gubernacular traction. The penetrating power of the unstriped muscle of the gubernaculum is of importance, but it develops in the canal, beneath the peritoneal ridge derived from the inguinal fold, *i.e.*, is in the main sessile and not effective for exerting downward traction. It is not attached directly or even indirectly to the testis, as the upper attachment of the caudal ligament is to the epididymis and not to the testis. Bramann, however, says it is attached at the 4th month.

The striped muscle in connection with the gubernaculum ultimately forms the external cremaster. It does not favour descent by any means: indeed any action, if it really occurred in fœtal life, would cause ascent of the testicle, as it does in adult life. The external cremasteric fibres passing into the lower part of the gubernaculum form the ascending cremasteric fibres, and are analogous to the conus inguinalis of rodents.[1] The internal cremaster is unstriped muscle round the vas and vessels, and in the tunica vaginalis propria.

Thus while the cremaster fibres advance at first at the apex of the penetrating gubernaculum, their function is in relation to the adult cord and testis.

Minor factors may help descensus. Thus Eberth mentions intestinal pressure, and Bramann considers the distended sigmoid had some influence in depressing the left testis. Increased inclination of the pelvis has been considered to have an influence by altering the direction of the inguinal fold favourably for traction. The lengthening of the cremaster has been supposed to exert traction, but all these, if not wrong, are insignificant, so that Eberth is right in his contention. "Vielmehr scheinen aktive und komplizierte Wachstumsvorgänge bei der Verlagerung des Hodens die Hauptrolle zu spielen."

I agree with this, and would minimise even the ultimate shrinking traction urged by Frankl, were it not for its apparent action in ectopia testis.

[1] Lockwood in his work rightly says that "the ascending cremaster of the human embryo is so trivial that perhaps it ought to be looked on as a mere survival of a muscle which in some of the lower animals is more active and better developed" (*op. cit.*, p. 108). Klaatsch's work on the conus inguinalis confirms this.

V. The Phylogeny of the Parts concerned in Descent of the Testes and of Descent Itself.

The phylogeny of an organ or developing process in a plant or animal is the history of its occurrence and development in some division of the animal kingdom, usually in the phylum or class of the animal or vegetable world to which it belongs. We are specially concerned just now with the phylogeny of the anatomical structures or organs involved in testicular descent in mammals, and with the phylogeny of the process itself. Up to this point we have been considering their ontogeny, *i.e.* their development in special animals or species. From the fact that we have, in this question of descent of the testes, to consider the organs and descent in the various species of the mammalia so far as known, as well as the embryology in many of them, the problem is a most fascinating one, and will repay careful consideration.

I purpose therefore to state the main facts bearing on the phylogeny of our subject. Some repetition is unfortunately unavoidable, especially as some of the structures, for instance the gubernaculum and cremaster, are joined with one another anatomically and functionally.

The organs concerned are the *scrotum*, *gubernaculum*, *cremaster*, and *inguinal canal*, and we shall consider these first, and then the *process of descent* itself.

The *scrotum* is a temporary or permanent pouch or sac for the testes. In the former instance, in certain mammals, at the rutting period, the testes pass back into the abdominal cavity, to re-enter the scrotum after the rutting period is over: in the latter case in other mammals they remain permanently in the scrotum when once they have passed in. In some of the latter, the processus vaginalis may be closed or open.

In the *monotremata* we start from "bed-rock," inasmuch as in these, the lowest of known mammals, there are none of the structures present whose phylogeny we are considering; they appear at first sight to come into the existing mammalian species *per saltum*, first in the marsupials, but the significance and accuracy of this requires to be carefully scrutinised. In the *marsupials* the scrotum is, in its position and development, the analogue and also the homologue of the female mammary pouch. In some males, apparent rudimentary mammary skin folds remain, but these are merely the folds after the scrotum has separated from its epidermic bed. The development of the mammary pouch in the female is by a passage backwards and outwards of the deep and superficial layers of the epidermis into the subjacent connective tissue: the connective tissue beneath the epidermis is not snared in. In the development of the marsupial scrotum

the deep layer of the epidermis passes back and in and snares in the connective tissue which forms the site of the future interior of the scrotal sac. The amount of superficial epidermis passing in is slight, but its ultimate desquamation frees the scrotum, superficially embedded at first as it is in the epidermis, and allows of its pendulous character. In most marsupials the mammary pouch has its opening above for obvious reasons, but in one at least, Katz figures the aperture as opening below with a sphincteric muscular arrangement of evident utility. This position of the aperture is of importance as showing an intermediate stage relative to the openings of the mammary pouch and its analogue. In regard to the muscular arrangement of the mammary pouch, the round ligaments act, according to Cunningham, as a compressor mammæ, while the sphincter is developed from the subcutaneous unstriped muscle.

The mammary pouch, then, may have a caudal or cephalic aperture, but the scrotum, its analogue and homologue, has its aperture cephalic and communicates up to its later stages with the peritoneal cavity (open processus vaginalis), has the testis ultimately in it, and then usually becomes shut off from the peritoneal cavity by the closure of its processus vaginalis. In *rodents and insectivora* the scrotum is a shallow pouch in the abdominal wall in the region of the inguinal teats, the cremaster sac or pouch. When the testes are in the abdomen in the adult, the transversales and internal oblique muscles project into the inguinal fold, thus forming a conical projecting eminence in the peritoneal cavity—the inguinal cone (conus inguinalis) of Klaatsch, who first drew attention to it. The nature and functions of this "conus" will be considered presently.

In *rats* the scrotum is lower down, towards the perineum, and finally in higher mammals it becomes the pendulous, sac-like scrotum.

The following summary gives the scrotal conditions known to us in the chief species of the mammalia. For convenience, I add in this summary the main facts as to position of testes, the gubernaculum, and the cremaster. The conditions, however, vary very much: there is no gradual gradation but an undulating one, and we must therefore conclude that variation is still going on in regard to these organs and to their descent.

Scrotal Conditions and those as to Gubernaculum and Cremaster in the Chief Orders of Mammalia (mainly from Frankl).

Monotremata.—Testes abdominal; no scrotum; no inguinal fold; no cremaster. Echidna shows ligamentum testis joined to vas deferens.

Marsupialia.—Suprapubic scrotum with processus vaginalis closed; mesorchium broad and four-angled; inguinal fold well developed.

Edentata.—Testes abdominal; position of testes really varies; may be primary

abdominal, subintegumental, or secondary abdominal; no scrotum; no inguinal fold; cremaster has transverse and internal oblique fibres; no conus; in *Dasypus sexcinctus*, inguinal fold marked and runs to equivalent of processus vaginalis, ending in its fundus; in *Dasypus noremcinctus*, short conical cremaster sac from internal and transverse below aponeurosis of external oblique.

Cetacea.—Testes primary abdominal and no inguinal fold.

Proboscidea.—Testes abdominal.

Rodentia.—Testes in scrotal pouch, but return to abdomen at "rutting"; cremaster from transverse and internal oblique, and forms "conus inguinalis."

Insectivora.—Much as in rodentia; have conus inguinalis, but not always; testes in some, abdominal, and no descent; in others, abdominal, and return to scrotum after rutting.

Chiroptera.—Testes return; conus present; cremaster from transverse and internal oblique.

Pinnipedia.—Testes extra-abdominal, subintegumental in inguinal canal; shallow cremaster sac from transverse and internal oblique; no scrotum; no return of testes; in *Phoca Vitulina*.

Carnivora.—Show beginning involution of processus; cremaster from transversus.

Artiodactyla.—Processus vaginalis narrow; cremaster from internal oblique.

Perissodactyla.—More primitive conditions; processus vaginalis wide open; traces of inguinal ligament even in adults; cremaster from internal oblique and well marked.

Prosimiæ.—(Lemurs) Processus vaginalis narrow; cremaster from internal oblique and transversus (mainly).

Primates.—Conditions very varied (*v.* Frankl, pp. 186–187), from simple to complex.

The facts are too varied to give any definite results, but some points are interesting.

The *monotremes* show the most rudimentary conditions. The *marsupials*, however, approach man in having definite scrotum, usually closed processus vaginalis, well-marked gubernaculum, very definite descent of testes in embryo, with a preformed inguinal canal.

Their scrotum shows clearly the most primitive type of scrotum, being evidently mammary in its nature and suprapubic in position. Its cremaster is derived from the transverse and internal oblique muscles as in man, and its fasciæ are much the same. Its gubernaculum, however, is not the specialised scrotal fibres of man, but consists of well-marked abdominal fibres which are normally rudimentary in man. The transition from monotreme conditions to marsupial ones is thus extraordinary.

We may put down, abdominal testes; absence of, or rudimentary scrotum; open processus vaginalis; return of testes to abdomen at "rutting," all as characteristic of a low position in mammalia; while permanent scrotum, especially if perineal; closed processus vaginalis, are all evidence of a high position. Exceptions, however, are plentiful, and in the *edentates* and *primates* we find almost all forms.

Primitive or comparatively primitive conditions are found in *Monotremes, Edentata, Proboscidea, Cetacea, Rodents, Insectivora, Chiroptera, Pinnipedia, Carnivora;* while in the *Artiodactyla, Perissodactyla, Carnivora, Prosimiæ, Marsupialia,* and *Primates* the arrangements are more advanced and finally culminate in the most advanced type as found in man.

Klaatsch has shown that in many mammals the site of the future scrotum is marked out by a certain area of skin, the area scroti, evident both by its naked-eye and microscopic character. The hairy covering is less marked; the small hairs arise from projections due to elevations of the cutis which possess a thin epidermic covering. Its most characteristic microscopic structure is a layer of unstriped muscle, ceasing abruptly at the edge of the "area." In the middle line the "areæ scroti" coalesce. The full phylogeny and nature of the scrotum will be best taken up after the gubernaculum and cremaster and conus have been considered.

The Gubernaculum, Cremaster, and Conus Inguinalis.—I need not recapitulate the facts as to the gubernaculum, but merely point out the constancy of its type in all mammals above monotremes. Thus its lower end is always in a mammary area, its upper at the Wolffian duct. It is not connected directly or indirectly with the testes. Its origin and insertion, in constant relation to primitive structures, viz, the mammary area and Wolffian duct, explain its almost uniform structure and relations in all species. In all, it acts as the active agent, with peritoneum and cremaster, in preforming the inguinal canal.

The *cremaster* is quite constant in all mammals above monotremes, and is derived, in almost all mammals, from the internal oblique and transversalis muscles. In front of it, as it passes in with the peritoneum and gubernaculum, lies the aponeurosis of the external oblique. Occasionally only one muscle forms the cremaster. In the marsupials the pyramidalis is well developed, but takes no part in the cremaster, as the gubernaculum skirts its outer edge, as it does that of the rectus. The great function of the cremaster is in the adult, as I have already stated, and it takes no part in causing the descent of the testes. It grows down with the inguinal fold, but how far, actively or passively, is difficult to say.

Conus inguinalis.—This is an important modification of the cremaster and gubernaculum found in rodents and insectivora, and first described by Klaatsch. I have found what appears to be its representative in marsupials, but in them it plays no part in changing the position of the testes. In rodents it can be seen as a cone projecting into the abdomen from the scrotal site, and it consists of fibres from the internal oblique and transverse muscles, passing into the inguinal fold. When the testes are in the scrotal

inguinal pouch, the "conus" runs from the inguinal fold to the base of the scrotum. The muscular fibres must have grown into the fold (Wiedersheim). They do not draw the testes into the scrotal pouch, as the direction of their fibres prevents this; nor can they draw it out. It would be absurd to consider them as drawing the testes at one time into the abdomen and at another time into the scrotum. Probably the best idea is to consider the cremaster fibres of the conus as first growing up into the inguinal fold to form the conus. Then they grow into the scrotum after rutting. Thus, at rutting, the conus develops or grows into the inguinal fold and by its shrinkage or involution after rutting, and by accommodation, the testes resume their scrotal position. This is the most probable and consistent explanation in the present state of our knowledge, but serial sections at the various stages would be needed to confirm or reject it.

Klaatsch figures a conus in the human embryo, and Eberth does so too.

Thus the cremaster has its share in the function of developing the inguinal canal and the cavity of the scrotum, and ultimately, in man for instance, forms a muscular incomplete covering to the cord and testes. It is a valuable supporting constituent in a pendulous organ, and has a probable function in preventing dilatation of vessels in the cord and testes. Its action under voluntary impulses in man is known and is well figured by Wiedersheim, but in the descent of the testes into the preformed inguinal canal it has not as yet been shown to play any direct part.

Statement as to Nature of Relations of Scrotum, Gubernaculum, and Cremaster.—In man the scrotum develops partly in the perineal region and partly above this, and the question now arises: Is this region and that of the labia majora in the female related phylogenetically to the suprapubic region of the marsupial or to the inguinal in rodents where the scrota respectively develop? If so, it would enable us to make the consistent statement that *the gubernaculum and round ligament, and with them for a certain distance the peritoneum and cremaster, are developed in relation to a mammary region in all mammals*, thus extending the striking generalisation first made by Klaatsch in his suggestive paper. Developmentally the labia majora and scrotum are due to an extension downwards and backwards from an area contiguous to and blending with the inguinal region. We have seen that the developing gubernaculum abuts on the abdominal wall at this point before it begins to penetrate, and thus the scrotal or labial skin is practically a pendulous extension of the inguinal.

The nerve and vascular supply to the scrotum bear this out. The upper part of the scrotum is supplied by nerves and blood-vessels common to the inguinal region. Cooper states that "(1) a branch of a lumbar scrotal nerve . . . . divides into numerous branches which supply the skin of the

groin, scrotum, and skin of the root of the penis; (2) the external spermatic nerve . . . . is distributed to the cremaster and the cellular tissue of the scrotum . . . . sends a branch to the skin of the groin . . . . The perineal nerve supplies the lower part of the scrotum."

A striking confirmation of this generalisation would be an abnormal teat or mamma on the scrotum or labium majus. I ventured to predict to a scientific friend that this would be found, and finally came on a reference to a mamma on the labium majus in Bateson's invaluable work on *Materials for the Study of Variation* (p. 187), where he quotes Harting, *Ueber einen Fall von Mamma accessoria*, the mammary structure of the gland being verified microscopically. I have said that Klaatsch has drawn attention to the fact that the gubernaculum and round ligament end in a mammary area, and I have confirmed and extended this. This would lead one to the conclusion by Klaatsch that the changes in the mamma induced by pregnancy are analogous to the changes in the conus inguinalis of the resting and rutting male. One might indeed look back, as Klaatsch suggests, to a primitive period when the young were suckled by both parents, and that then the differentiation took place which ended in the predominance of the mammary function in the female, with a round ligament equivalent to the developing gubernaculum only, and a rudimentary inguinal canal; while in the male the mammary function became rudimentary, and the gubernaculum initiated the changes in the abdominal wall, which not only gave the inguinal canal, but also the descent of the testes. This, however, is very speculative. I agree with Klaatsch in his views as to the mammary area insertion of the gubernaculum, but he has not pushed his most interesting theory far enough.

Let us apply it to the marsupials. In the male we have a scrotum topographically and developmentally equivalent to the mammary pouch; it contains the testes. In the female we have a mammary pouch with the round ligament, the analogue of the gubernaculum, ending in it, in relation to the mammary gland. One usually looks on the mammary pouch as only a pouch for the mamma, and for the young marsupial. To make it exactly equivalent to the male scrotal arrangement, it should, however, contain the ovary. It does not; but if we go back to the monotreme echidna, we find, as Haacke has shown, that it carries its egg—the product of the ovary—in a pouch developed for it at the time, a pouch large enough to hold almost completely a gold watch. *The mammary pouch therefore is primitively the egg or ovarian-product pouch just as the scrotum is the testicular pouch.* Thus in all mammals above monotremes the developing gubernaculum joins the lower end of the primitive Wolffian body to an area of skin which is primitively an ovarian-product or testicular pouch—a

mammary area; and when it loses the fœtus-carrying functions (as it does in all above marsupials) retains in the female the mammary function, and in the male the testicular pouch function. The development of the inguinal fold and cremaster thus begins primitively in rodents in Klaatsch's inguinal cone, and develops to the more perfect gubernaculum of higher mammals. The active agent in the gubernaculum is the unstriped muscle; thus the peritoneum only forms a shallow processus in the female processus; it is the unstriped muscle that mainly forms the round ligament and preforms the inguinal canal.

The Inguinal Canal.—On this one can be brief, as much of its phylogeny is involved in the previous sections. There is no inguinal canal in the monotremes. It may be a shallow pouch (rodents, insectivora); a deeper canal, with its processus narrow or closed; a well-formed canal in the embryo, with closed processus (marsupials, carnivora, primates, man). Its line of evolution is thus, increase of depth in abdominal wall (its direction varying according to the position of the scrotum), and closure of processus. Its highest development is thus in man, but it is high, as already noted, in marsupials. The position of the inguinal fossa or canal, as the case may be, is determined by two factors: the direction of radiation of the gubernaculum fibres and the area of spread of a developing lymphatic centre. This is best seen in the marsupial embryo, but probably holds good for others.

Descent of the Testes.—There is no descent in monotremes, edentates, cetacea, or proboscidea. The first beginning is in rodents and insectivora, and there the descent is temporary and periodic after rutting. As we pass up the mammalian scale the descent becomes more marked, penetrating the abdominal wall and into a scrotum, inguinal, suprapubic, or perineal. While we use the term "descent" it must be noted that this is most marked in the abdominal phases; afterwards, when the testes are in the inguinal canal, the testes are relatively stationary, and growth of the inguinal canal is the active factor; descent again asserts itself if the testes reach the perineal scrotum; but if the scrotum is suprapubic, this last stage is really an ascent. We thus must always use the inevitable term "descent" with these reservations.

The Relation of Descent of the Testes to Haeckel's Law and to Mammalian Classification.—Haeckel's law (or rather the Müller-Haeckel law) is briefly stated as follows[1]:—Ontogeny repeats and condenses phylogeny in whole or in part—the development of the organs and their functions in man repeats and condenses in time and stages the parts of various organ-

[1] See Weismann's *Theory of Evolution*, vol. ii. p. 160; and also *Darwin's Life*, where Darwin claims this law.

ontogenies and their functions necessary to complete the ontogeny of the whole organism. In some stages, indeed, it gives what may appear an irrelevant reminiscence of its lower mammalian origin, as in the ascending cremaster fibres and temporary ascent of the testes.

This is a great generalisation, and is completely vindicated when we consider testicular descent in man. We cannot but accept this law, which points most probably to the continuity of the germ plasma as being at the root of the phylogenetic repetition in ontogeny. Let us consider the phylogeny of testicular descent. There is no descent in monotremes, in some edentates, in cetacea, and in proboscidea. The testes lie in a shallow inguinal pouch in rodents, and during rutting are abdominal from change in the "conus inguinalis," their cremaster: they are suprapubic in marsupials; perineal, and usually in a pendulous scrotum, in higher mammals.

The "ontogeny" of the process in man repeats in a few months of fœtal life (2nd to 8th) all these stages in the long phylogeny of the lower mammals. The testes are in the human male fœtus abdominal in the 2nd month (as in monotremes) at the peritoneal fossette, and afterwards a little higher in the 3rd to 4th month (as in the rutting of rodents); in the inguinal canal, *i.e.* subintegumental, at the 5th to 6th month, as in the rodents after rutting; and finally, perineal and scrotal at the 8th to 9th month.

In man the gubernacular fibres are scrotal, but in the pubic and perineal and inguinal rudimentary fibres we see a phylogenetic reminiscence of these conditions in marsupials and rodents. Those who believe in active gubernacular fibres in man as causing descent, regard these rudimentary fibres as aiding mechanically, like guy-ropes, the scrotal ones: but that the sessile or attached unstriped muscle of the gubernaculum can act "dynamically," *i.e.* cause transition, is erroneous; the true interpretation of the rudimentary fibres in man is seen in this, that they are an illustration of Haeckel's law.

In the development of our knowledge of testicular descent, indeed, it was really by a reversal of Haeckel's law that progress was made, *i.e.* the conditions in the lower mammals, a long-spun-out history, as it were, of what occurs in man, explained in the hands of the great early investigators —Hunter, Owen, Seiler—the more rapid and condensed stages in man— Haeckel's law was worked backwards by them.

But Haeckel's law can be used to bring back lost history, and supply stages in phylogeny we have lost; just as in his great periodic law as to the chemical elements, Mendeleëff rightly predicted that chemical elements would be discovered with certain definite atomic weights, and in approximate places in his scale, to complete the series.

Let us apply Haeckel's law to its logical extent. The monotremes and

marsupials are lowest in the mammalian scale, and yet the marsupial has a development of inguinal canal, and a condition after descent in many respects resembling the human male, and higher than that in rodentia, for example. So far, therefore, as the conditions we are discussing go, we cannot adopt a linear mammalian classification. Mammals must be classified in several lines radiating from an ancestor more primitively developed than the monotreme. If then we wish to speculate as to this ancestor, we must consider early ontogenetic stages of the region in which the descent process takes place, *i.e.* the rump end of human embryos. Such stages are to be found in the human embryo as shown by Keibel, when the entodermal cloaca[1] is formed, the urogenital ducts opening into a common closed chamber. Here the cloaca is closed in front by the cloacal membrane devoid of the mesoblast which afterwards develops. The yielding of this membrane gives the distressing "ectopia vesicæ" of the human adult, but this yielding and patency of the cloacal membrane may have been normal in some predecessor of the monotremes. The egg may have been incubated in the upper part of the entodermal cloaca, which lay beneath the region in which the abdominal egg-pouch afterwards developed. From such an hypothetical ancestor the mammals may have developed, and the subsequent grouping may be arranged as follows:—

After the hypothetical mammalian ancestor, the first group would include the monotremes and marsupials. Between these, however, there is, in their testes arrangement, a tremendous gap, one being testicondal, the other having a well-formed scrotum, a closed-off processus vaginalis, and a descensus only differing from that of man in having a suprapubic scrotum (the analogue and homologue of the mammary pouch), and a gubernaculum, the fully developed abdominal fibres of which are rudimentary in man. This gap must either have been gradually filled with slowly evolved and now extinct members of the marsupials, or we may hold that the inguinal fold came into mammals *per saltum*, in a way analogous to the "Mutation" theory of de Vries as to the origin of species. The existence of intermediate forms of testicular position and descent as in the higher mammals, as in rodents, however, negatives this.

The next diverging group would hold the edentata, sirenia, cetacea, proboscidea, and hyracoidea; then would come a parallel group of rodents, insectivora, and chiroptera; next the ungulata and carnivoræ, and finally the lemurs, anthropoids, and primates. Intermediate forms exist in the edentata, lemurs, and anthropoids, so that the groups are not sharply differentiated.

[1] This is better termed "penultimate gut," *pars penultima* of the primitive gut: the "tail gut" is then *pars ultima*.

I merely throw out the arrangement of classification according to the position of testes and the evolution of their descensus as a suggestion, and one that must be modified greatly as our knowledge increases.

My final conclusion is that the testis, appendix testis, and prostatic utricle, Wolffian body and its duct, the gubernaculum, the mamma, the external genitals, form an associated anatomical unit, the male urogenital and mammary unit—for shortness, the male genital unit; and in the same way, the ovary, epoophoron, Wolffian body and its duct, the round ligament, the mamma, the external genitals, are the female urogenital unit –for shortness, the female genital unit.

This is an important and convenient condensation of the relation of these organs, and in a future paper on Mendelian action on differentiated sex I go on to analyse the nature and significance of these units as to this question.

I may, however, sum up descensus testiculorum in terms of the male unit. The essence of the process is this:—The testis is united to a mammary area, at first by the testicular caudal ligament and the inguinal fold or gubernaculum, afterwards by the involuting caudal ligament and developing gubernaculum. The developing gubernaculum, with the aid of the cremaster and peritoneum, forms a pit or fossa for the testis in the rodentia: a more complete canal or more or less pendulous scrotum in higher mammals. By subsequent disproportionate growth of canal and testes, and finally (according to Frankl) by the involution and shrinkage of the gubernaculum, the testes in man become lodged permanently in the scrotum. I need not bring in intermediate stages in this summary. The progression in mammals is thus primary testiconda, secondary testiconda; finally, more or less of a descent of testes into a closed sac. The gubernaculum site of origin is primarily a Wolffian duct area and only indirectly, by means of the caudal ligament, testicular; the insertion always mammary.

What the reason of testicular descent is I do not know, but the gubernaculum always penetrates to a mammary area, and this area, in the human male, is finally a scrotal or labial one, and the formula of the progressive change in the relations of the testes, commonly called "descent" in all mammals, is this: *the gubernaculum always develops towards, and ends in, a mammary area, suprapubic, inguinal, perineal, scrotal. The testis appears to follow its guide—its canal-former—the gubernaculum, and the gubernaculum in marsupials certainly passes through the substance and skirts the edge of a developing lymphatic area.*

As the heading of this paper I have given three quotations from John Hunter's works.

The second one shows plainly that Hunter held that the testis came through the processus vaginalis.

In the first quotation we see Hunter laying the foundation of the subject of testicular position and descent. The gubernaculum, the term which has rightly become permanent in anatomy, he never uses in the sense of a "tractor," but always of a "rudder"—its true meaning. Here again, Hunter's teaching has been for long discarded, with disaster to accuracy and clear comprehension, and the gubernaculum has been credited with powers that the examination of serial sections shows to be illusory.

Hunter held the only possible view at that time as to the testis-covering, viz., that it was a peritoneal one in the abdomen and scrotum.

The last quotation is a remarkable one, and shows Hunter's unique powers as an unprejudiced observer. We see in the record of this fact, shadowed forth the modern view that the scrotum is equivalent to a mammary area, towards which the gubernaculum develops and the testis passes; and so we may fitly and finally say that in the investigation of this great anatomical and physiological question there is one observer who was at the beginning and is still in the van—John Hunter.

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Not only did Frankl clearly show the points as to the testicular covering detailed in my paper, but he quotes Hoffmann in the Hoffmann-Quain *Anatomie*, Erlangen, 1870, as saying, "Allein auch an der vordere Abtheilung des Hodens fehlt der Peritonealuberzüg, indem dieser nur mit einem schmalen Saum auf die hintere Abtheilung der Hodens in der Umgebung des Nebenhodens sich erstreckt, fast genau so wie Waldeyer das Verhalten des Peritoneums zum Eierstock beschrieben hat. Der grossere theil des Hodens ist frei vom Bauchfell." This was written thirty-nine

years ago. *Beiträge zur Lehre vom Descensus testiculorum. Sitzbericht der K. akademie der Wissenschaften*, Wien, 1900, Bd. cix. Hft. i. A most valuable monograph in the comparative anatomy of Descensus.

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Haacke, W., "On the Marsupial Ovum, the Mammary Pouch, and the Male Milk Glands of *Echidna hystrix*," *Proc. Roy. Soc. London*, 1885, vol. xxxviii. p. 72. The Echidna egg found in the pouch was 15 mm. × 13 mm., and thus nearly exactly round.

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As this rare case is in a somewhat inaccessible form, I give a short résumé of it. The patient was under the care of Drs Dietz and Heydenrich at Nürnberg. She was 30 years of age, and had observed this labial tumour for several years. During the suckling of her infant a milky fluid oozed from an ulcerated surface. The whole tumour was about the size of a large goose-egg, and had a special isolated palpable part the size of a walnut. The mass was pediculated, the pedicle being about 1 cm. long. It was easily removed. Macroscopic examination showed mammary structure, and a flattened-out teat with small openings was found. The fluid had fat globules. Without giving further detail, it may be said that the evidence of its mammary nature was absolutely complete.

His statistics are interesting. In 63 cases of accessory mamma he found 55 in women, 11 in men. In the women, 29 had one accessory mamma, 25 had two, and 3 had one. Of the 29, one was mammary, three in the groin, one on the back, one on the thigh, two above the navel, one on the axillary line, two in the axilla, eighteen on the breast.

Such cases are not uncommon. I have in my museum drawings of two specimens of double nipple and a cast of the axillary mammary or milk secretory condition to which Champneys has drawn attention.

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This represents the chief literature consulted. It is, however, fully given by Frankl and Klaatsch.

THE PHYSIOLOGICAL DESCENT OF THE OVARIES IN THE HUMAN FŒTUS.

By D. Berry Hart, M.D., F.R.C.P.E., F.R.S.E., *Lecturer on Midwifery and Diseases of Women, Surgeons' Hall, Edinburgh; Hon. Fellow, American Gynæcological Society; Carnegie Research Fellow.*[1]

Little attention has been paid to this subject, evidently for the reason that the normal descent does not go below the immediate vicinity of the brim of the pelvis, and that it is dwarfed by the more complicated question of the descent of the testes. The permanent position of the ovary, however, is of importance, as even after the ascent due to pregnancy it sinks normally to the same level. There are two other points of interest in addition, viz.: that while the ovary is never normally in the inguinal canal or labium majus, these are normally canalised to a certain extent by the round ligament, the analogue and homologue of the gubernaculum; abnormally, also, the ovaries may imitate the normal descent of the testes, and be permanently, usually on one side only, in the inguinal canal or even in the labium majus—the so-called hernia of the ovary. The question of their normal descent is thus of some scientific value. As two of the marsupial embryos I recently examined were female, with the ovaries developing on the Wolffian bodies, and as the round ligaments had penetrated the abdominal wall, I was able to trace the process to a certain extent in them, and also in two human embryos at the 5th to 6th week (h. to h. measurement, 31 mm.).

I shall therefore consider: (1) The development of the round ligament and of the mammary pouch: and (2) The descent of the ovaries.

I. The Development of the Round Ligament and of the Mammary Pouch.

On examining one of the marsupial embryos in serial section from above down (the other confirmed the findings), the first thing that struck one in the suprapubic region was the presence of an oblique chain of lymphatics lying in front of the pyramidalis muscle on each side; and lateral to the developing mammary pouch. They crossed the line of the future inguinal

[1] From the Laboratory of the Royal College of Physicians, Edinburgh.

canal, were not so marked as in the male, and formed, as in them, oval canals lined with thin spindle-celled fibres and round nuclei, the latter projecting into the lumen slightly. The connective tissue and unstriped muscle between and near these sinuses was markedly increased. A distinct bundle, part of the round ligament in the abdominal wall, formed a curved band in the lymph site round the outer side of the pyramidalis and marsupial bone. This appeared as a cone-shaped projection, probably the analogue of Klaatsch's inguinal cone, bulging the peritoneal lining of the abdominal cavity in the neighbourhood of the point of entrance of the inguinal fold to be presently described. The round ligament had thus penetrated the muscular fibre of the internal oblique and transversales muscles, and had now in its substance striped muscular fibres. In its substance, too, can be seen some of the lymph sinuses, and near the abdominal wall there is a distinct cavity with a ridge on its inferior aspect. The round ligament here is bounded by the connective tissue of the abdominal wall, and nothing at present analogous to the tunica vaginalis except the cavity and ridge just alluded to is to be noted.

In one specimen, muscular fibres traceable to the internal oblique can be seen in the substance of the ligament, and loose connective tissue separates it from the denser connective tissue to the outside.

I have already described the nature of these lymph-sinuses in the male in a previous paper on the descent of the testes. They are the sinuses of the lymphatic system developing from the vein in the groin, budding off from them as Dr Florence Sabin has already described, and I need not recapitulate this description therefore, except to say that the chains as seen on transverse section lie one on each side, in front of the pyramidalis, and each is joined to its fellow by a band of connective tissue as in the male. They form a ladder in the subcutaneous tissue running obliquely from below up, in this case above the level of the top sections which lay below the rib-level. The *round ligament* is at first the inguinal fold, *i.e.* before it begins to penetrate the abdominal wall. In two human embryos at the fifth to sixth week one sees the inguinal fold meeting the abdominal wall with the internal oblique and transversales muscles and tendon of the external oblique in front, but as yet there is no penetration. In one of these, prepared by Gulland in serial section, the lymph-sinuses are seen well developed where the round ligament meets the wall (fig. 2). They thus bear out the relations of lymph-sinuses to gubernaculum already shown in the case of the marsupials.

In the part of the round ligament nearer the Wolffian body wall the unstriped muscle was well developed, but nearer the abdominal wall its place was taken by round-celled connective tissue. One formed the impres-

sion that unstriped muscle was radiating into the round ligament from a posterior source. On tracing from below up the serial sections of one of the marsupial female embryos, which were at a later stage of development than the human specimens, so as to ascertain the relations of the round ligament as it passed through the abdominal wall, the appearances were as follows:—One came upon first the inguinal fold penetrating the abdominal wall (fig. 1) and forming a shallow peritoneal pouch. In this penetration, however, the peritoneum soon ceases to be active, but the unstriped fibres

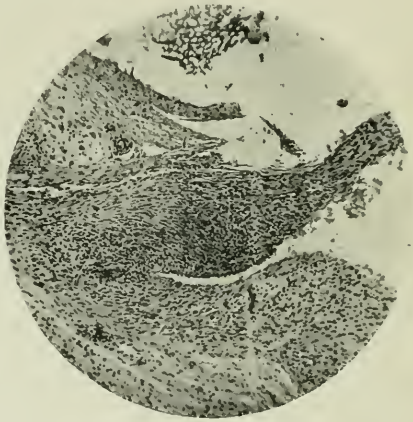


Fig. 1.—Shows inguinal fold, future round ligament penetrating, and minor participation of peritoneum in it. ($\frac{8}{1}$.)

grow on and up, and mingled in them are the striped cremasteric fibres, not advancing with them *pari passu*, and thus not forming part of the advancing wedge we see so well in the development of the cremaster associated with the male gubernaculum. The unstriped muscular bundle arrives at the lower end of the mammary pouch, and appears to end in the round-celled connective tissue in which the mamma afterwards develops.

In adult marsupials, as Katz's specimens show, the round ligament, or cremaster as Katz terms it, runs in front of the pouch well above its deep part; and thus, after the stage I have just described, the pouch must deepen below the level of the cremaster insertions.

The round ligament, like the gubernaculum, invades the lymphatic area

in the groin; and this may determine its line of growth, as it appears to do that of the gubernaculum in the male. In its subsequent growth the round ligament does not pass through the stages the gubernaculum does, and thus the ovaries have only normally a descent in the abdomen, and not any further descent comparable to that of the testes.

The adult round ligament is very distinct. It is really equivalent to the developing gubernaculum prior to the descent of the testes, and not to the developed gubernaculum: it remains in the stage of the developing

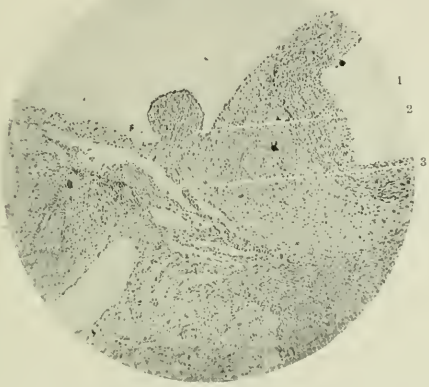


Fig. 2.—Trans. section, female embryo, 5th-6th week. ($\frac{5}{1}$".)
1, round ligament; 2, bladder; 3, lymph-sinuses.

gubernaculum, and grows with the growth of the uterus. Not only that, but the ovarian ligament, the analogue of the caudal ligament of the testis, remains as a well-marked structure. We see on the anterior aspect of the broad ligament in the adult the origin of the round ligament very well. It arises at the junction of the uterus and tube in front, and is thus opposite the uterine end of the ovarian ligament. Its origin thus marks the spot corresponding to the origin of the gubernaculum in the early male embryo. The gubernaculum arises from the epididymis, where the globus minor is continuous with the vas deferens (Frankl), and we thus can make more precise the nature of the parovarium or epoophoron. The parovarium comprises the vertical tubes and the longitudinal tube which represent the

epididymis, and has a free longitudinal duct which ceases at or near the origin of the round ligament. Thus this longitudinal tube must end at a point corresponding to where the globus minor and vas deferens in the male meet—any part of this duct remaining at the side of the uterus or in the vagina is equivalent to part of the vas deferens. The important point is that the epoophoron with its duct found normally in the broad ligament is a definite part of the Wolffian body and its duct, *i.e.* comprises the analogue of the epididymis, and a part of the Wolffian duct down to the globus minor. So far as I know, this fact has not been pointed out.

We see, therefore, that the origins of the caudal ligament of the testes and that of the ovarian ligament are analogous.

As Hill's specimen of the embryo male pig shows,[1] the caudal ligament has its origin near the testis' caudal end, at the junction of the epididymis and vas deferens, or beginning of the duct of the epididymis. It ends on the upper and inner surface of the genital ducts opposite the point where the gubernaculum arises. The ovarian ligament springs from the uterine end of the ovary, but whether its origin is exactly like that of the testicular caudal ligament is not accurately known. It ends at the junction of tube and uterus opposite the point where the round ligament springs. They are thus equivalent in every respect.

The *mammary pouch* develops in a very simple manner (fig. 3). In the suprapubic region, the deep and superficial epidermis passes back, laterally and down, to form a solid, flattened epidermic cone, the superficial cells being central. This cone penetrates the band of unstriped muscle joining the inner ends of the superficial lymph sinuses. In its upper part, this cone or plug is continuous with the free abdominal surface, but nearer the pubes it becomes snared off. It is evident that desquamation of the epidermis gives a pouch with its opening above. From the posterior aspects of this pouch the mammary structures bud out, but my specimens show only the early buds—Bresslau has, however, worked this out. The unstriped muscle of the intersinus band thus comes to lie in front of the pouch, where the epidermic plug is buried in the abdominal wall.

The development of the mammary pouch is thus related to lymphatic sinus development, inasmuch as it takes place between the inner ends of the sinuses: on the other hand, the round ligament and gubernaculum develop along the outer edge of these sinuses.

The chief conclusions may therefore now be summed up as follows:—

The round ligament of the adult human female is equivalent to the gubernaculum in the male embryo at the stage when the preformed inguinal canal has been made by the latter and the testes are still abdominal,

[1] *Vide* paper on "Descent of Testes," Part I., fig. 8, p. 253.

and not to the fully developed gubernaculum when the testes are descending. It has thus no stage of involution.

The mamma, round ligament, Wolffian body and its ducts, with the ovary, form a connected system—an anatomical unit in the body—analogous to what I have already described in the male. It comprises mamma, ovary, tubes, vagina, urogenital sinus, hymen, external genitals, epoophoron, upper part of Wolffian duct and lower end of Wolffian duct. This is the female urogenital and mammary unit—for shortness the female U.G.M. unit—and will

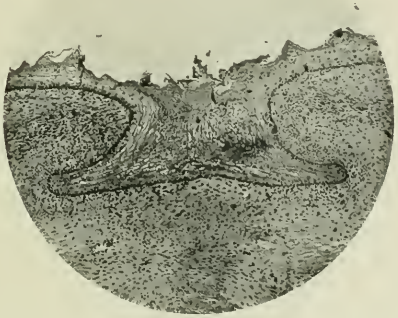


Fig. 3.—Epidermis, on anterior abd. wall, passing back and in, to form mammary pouch. ($\frac{5}{9}$.)

be analysed along with the male U.G.M. unit in its elements and significance in a subsequent paper on "Mendelian Action on Differentiated Sex."

II. The Descent of the Ovaries.

The ovaries develop like the testes on the inner and anterior aspects of the Wolffian bodies (fig. 4).

There is a well-marked mesovarium, better marked than the primary mesorchium. They lie at first in the lumbar region, but about the 5th to 6th week their position is lower and behind the genital cord, where the mesovarium is well marked. In the adult female they lie on the posterior aspect of the broad ligament, on the lateral pelvic wall, in front of the sacroiliac joint, immediately below the iliopectineal line, and with the

ureter curving behind. Their descent is thus purely from the abdomen into the upper strait of the pelvis. The question may be asked why the ovaries descend no further. It seems at first sight that they may be arrested by the infundibulo-pelvic ligament, and also because the well-developed uterus and broad ligament lie in front, and may block further descent. The ovaries may, however, descend as far as the inguinal canal or into the labium—so-called hernia of the ovary—but this term masks the real condition, viz., that it is a descent analogous to the stages of the

FIG. 4.—Six weeks' human embryo. ($\frac{5.0}{1}$.)
1, ovary; 2, mesovarium; 3, Wolffian body.

descent of the testes. The main cause is the normally arrested development of the round ligament. This, as already pointed out, is the equivalent of the stage of the gubernaculum while the testes are still undescended. There is thus nothing more remarkable in normal descent of the ovaries in the adult than there is in the position of the testes with the gubernaculum in a condition similar to that of the round ligament.

The round ligament passes into the solid labium majus, and this structure, the equivalent of a half scrotum, is a mammary area, shown, not only by the vascular and nervous supply of its upper part, which is, in common with that of the inguinal region, undoubtedly a mammary area, but also strikingly proved by the labial mamma Harting has so fully described. The round ligament thus ends in a mammary area and connects

the ovary through the ovarian ligament with it, just as the gubernaculum indirectly by means of the caudal testicular ligament joins the testes to a mammary area. It thus fulfils for the ovary the anatomical generalisation I have made for testicular descent, *i.e.* the round ligament passes to a mammary area, modified only by the fact that it does not pass through the full development the gubernaculum does.

We have not the anatomical detail of the descent of the ovary that we have of the testes. The difficulty here is the want of material, but in large maternities like those in our chief medical centres excellent investigations could be made, and these would throw light, not only on some points in testicular descent, but also on those of excessive ovarian descent usually imperfectly described as hernia. They are analogous to the cases of ectopia testis, but it is paradoxical and yet correct to say that inguinal and labial ectopia ovarii are normal stages for the testes, while pelvic ectopia testis is analogous to the normal ovarian position.

For literature see previous paper on "The Physiological Descent of the Testes," and Hertwig's *Lehrbuch*.

X.—The Structure of the Reproductive Organs in the Free-Martin, with a Theory of the Significance of the Abnormality.

By **D. Berry Hart**, M.D., etc., Edinburgh.* (With Two Plates.)

(MS. received November 15, 1909. Read November 22, 1909.)

The apparent prodigality with which Nature provides for the reproduction of plants and animals has a marked exception in the case of sterile organisms, often produced on a large scale, in which every function except that of propagation may be carried on in a quite perfect manner. The cases of the sterilisation of an organism are best seen in insects, but I wish at present to consider its occurrence high up in the animal kingdom in the well-known case of the Free-Martin, an apparent sterile cow born co-twin with a potent bull.

The nature of the free-martin is considered one of the unsolved problems of anomalous sex. John Hunter, who first drew attention to it, speaks of it as an unnatural hermaphrodite. "Hermaphrodites may be divided," he says, "into two kinds, the natural and the unnatural. The natural hermaphrodite belongs to the inferior and more simple genera of animals, of which there is a greater number than of the more perfect: just as animals become more complicated, have more parts, and each part is confined to its particular use, a separation of the two necessary families for generation has taken place" (*op. cit.*, p. 46). Sir James Simpson, in his well-known paper, after enumerating the paradoxes in the knowledge at that time of the free-martin, says in conclusion: "The whole series of circumstances, when considered in conjunction with each other, seems to form, in relation to the origin of malformations, one of the strangest and most inexplicable facts to be met with in the study of anormal development" (*op. cit.*, p. 835).

Spiegelberg looked on the free-martin as a transverse hermaphrodite, and Geddes and Thomson say: "No theory has yet explained the facts of this case" (*op. cit.*, p. 41).

The explanation I wish to bring forward is based on the views expressed in a previous paper on "Mendelian Action on Differentiated Sex."

I must first describe the free-martin, next consider the facts known as to it, and then give my view as to its anomalous sex-condition.

* From the Laboratory of the Royal College of Physicians, Edinburgh.

The free-martin is a sterile twin, usually co-twin with a potent bull.* It has, in this case, the lower part of its genital tract to the naked eye like that of a cow, the upper part defective, and is usually considered as a cow sterile from incomplete development of its upper vaginal and uterine tract.

John Hunter described three specimens:—

(1) *Mr Arbuthnot's Free-Martin* (*op. cit.*, p. 52).—This animal was seven years old; went with the cows and bull; never showed any desire for either. The external parts were rather smaller than in the cow; the vagina was contracted above the urethral opening, becoming continuous with a small canal; uterus with two horns, two ovaria, and two testicles: vasa deferentia to the testicles; the left one did not come near the testicle, the right one only came near to it but did not terminate in the epididymis. They were both pervious, and opened into the vagina near the urethra. Vesiculæ seminales were present, smaller than in the bull; "the ducts opened along with the vasa deferentia." "This was more deserving of the name hermaphrodite than the following, for it had the mixture of all the parts, although all were imperfect."

(2) *Mr Wright's Free-Martin*, fig. 1, five years old (*op. cit.*, p. 53).—This animal was more like the ox or spayed heifer than the bull or cow. The vagina had a blind end near the urethral orifice; a two-horned uterus; testicles, and not ovaria; this based on their size nearly equal those of a bull. Vesiculæ seminales opening into vagina, but nothing like vasa deferentia; a clitoris was present. "This animal cannot be said to have been a mixture of all the parts of both sexes."

(3) *Mr Well's Free-Martin* (*op. cit.*, p. 54).—Three to four years when killed, more like a heifer; no desire for male. Beginning of vagina as in cow, but obliterated beyond the urethra, although a solid part was continued. Two horns and two ovaria; vas deferens in interrupted portions. Between vagina and bladder the vesiculæ seminales, and between them the termination of the vasa deferentia. "This could not be called an exact mixture of all the parts of both sexes, for here was no appearance of testicles (*op. cit.*, p. 54). He thus considered Arbuthnot's case to have testes and ovaries; Well's case to have ovaries, and Wright's case testes.

Sir James Simpson, in the paper already quoted on "The Alleged Infecundity of Females born co-twin with Males," states as his conclusions that the human female co-twin with a male is as likely to be fertile and have as many children as the normal woman not a twin (*op. cit.*, p. 835).

* The reason for this limitation will be seen afterwards.

The third paper I have now to consider is one by Professor Otto Spiegelberg, the late distinguished obstetrician of Breslau, who examined the parts carefully by the naked eye and microscopically in a free-martin full-time calf, and threw great light on the nature of the free-martin by showing that it was *an imperfect male calf and not an imperfect female calf.* Spiegelberg had, of course, the advantage over Hunter and Simpson of the more advanced knowledge of his time in microscopical technique and embryology. Rueff also noted in two cases that the sexual glands were testes (1851), and Gurlt, in 1832, states the same.

Spiegelberg's cases were two in number, and are briefly as follows:—

Case 1.—This was one of twin calves killed a few days after birth. Externally, both calves seemed normal. The male had the testes in the scrotum, the internal genitals were quite normal. In the apparent female there were no traces of uterus, tubes, and ovaries (*nichts von Uterus, Tuben und Ovarien*); instead there was a special complex of organs, which, beginning a little distance from the kidneys in a peritoneal fold, ran down between the rectum and bladder (*vide* Pl. I.). There the external genitals are opened from the front and turned to the side. Vulva and clitoris normal; the former passes into a narrow canal $1\frac{1}{2}$ inch long and 1 inch broad, with smooth walls, on whose anterior wall the urethra opens, and is accordingly the vestibule. No trace of the openings of Gartner's canals was evident. At the apex of the canal is an opening the size of a linseed, with no bridge of hymen, through which one can carry a sound up and back into a cavity scarcely an inch long. The wall of this cavity is $\frac{1}{2}$ inch thick, with connective tissue and transverse muscular fibres. Laterally from this rudiment of the vagina there run up two hollow processes, blind above, about 15 lines long; below, they unite with the wall of the cavity, and have the knob of the sound *c* between them; above, they end free in the connective tissue; both have a narrow lumen, blind at both ends. Between them lie two cords close to one another, *e* springing from the upper wall of the vagina; the left, 16 lines long, loses itself in the peritoneal fold; the right runs tortuously up as a fine thread, ultimately to a structure *g*. Both cords form a relatively wide canal, from which a few drops of white, slimy fluid can be squeezed. The canals are closed at both ends; *f* is solid. At the upper end of this arrangement there lie on each side two structures, at first glance the sexual glands. The vulvar canal is thus vestibule or female urogenital canal: the cavity *b* is rudimentary uterus; *d d* are rudimentary vesiculæ seminales; *e* and *f* vasa deferentia; the double organs *g* and *h* not ovaries and testes, but testes and rudimentary

tubes, with a canalicular structure. The testes are thus seen at *g*, and at *h* rudimentary portions of the Wolffian body.

Spiegelberg considers this to be a case of transverse hermaphroditism in a bull calf, the upper part being male and the lower female in type. He summarises the literature carefully, and concludes that "if the twins are both female or of a different sex, their sexual organs are, as a rule, well developed; if both male, then frequently one is hermaphrodite. ("Sind die Zwillinge weiblich oder verschiedenen Geschlechts, so sind ihre Geschlechtsorgane in der Regel wohlgebildet; sind sie beide männlich, so ist sehr haufig das eine derselben ein Hermaphrodit" (*op. cit.*, S. 130).)*

Spiegelberg quotes Numan's article in the *Journal Vétérinaire et Agricole de Belgique par Brogniez, etc.*, année 1844, as a valuable contribution. This it undoubtedly is, as I have found on studying it.†

The summary given by Spiegelberg of Numan's conclusions is as follows:—

(1) If a cow has twins of different sex the female calf has almost invariably incompletely developed organs and is sterile.

(2) This fact based on observations has exceptions, and cannot be considered as indicating an exclusive law.

(4) The developmental error of the genitals does not happen exclusively to the female of similar twin couples, but occasionally to the male, and then the female is well developed: such cases are, however, rare.

(5) Multiple births are frequent in cattle, and, so far as the female is concerned, are to be considered the most certain and constant cause of sterility; the more so as the condition of the sexual organs

* *Case* 2.—In this case Spiegelberg found the twins of different sex and normal. They evidently came from separate zygotes.

† Numan's monograph was entitled "Verhandeling over de onvruchtbaren rundern bekend onder den naam van Kweewen," etc., met 23 platen; Utrecht, N. van der Monde, 1843. This monograph is not accessible in this country, so far as I have been able to ascertain. It is translated in the *Journal Vétérinaire et Agricole de Belgique* for 1844, and a copy of this was obtained by the Royal College of Physicians, Edinburgh. All the references to Numan's plates are given, but unfortunately not the plates themselves. Numan figures nine specimens, of which six are the same as Hunter's, but three show the sexual glands in the free-martin to be evident testes with the Müllerian element much less represented. A rudimentary preputial sheath is present in the latter, so that the animal is very like a bull. Numan terms the six like John Hunter's specimens "Heifer Free-martins," and the three remaining as "Steer Free-martins." He considers only the six as heifers, but all the nine are sterile bulls. He figures the vesiculæ seminales in all, but considers them in the six heifers as diverticula of Gartner's canal. In the points as to the sexual glands and vesiculæ seminales he is in error, but his whole monograph is a very able and valuable one.

which causes the anomaly does not as yet seem to have been observed in simple born calves. One finds, however, sometimes in such cases incomplete organs in a male.

The conclusion stated under (4) is the most novel, as it points to a free-martin with a sound female twin. It is of great interest theoretically in regard to Mendelism in this connection. The necessary modification of (1) will be seen presently.

As Hunter's specimens are still in the Museum of the Royal College of Surgeons, London, it was therefore a matter of great interest that the sexual glands should be examined microscopically. This has just been done under the direction of Dr Arthur Keith, the Curator, and by permissiou of the Council; and I am greatly indebted to Dr Keith for permission to examine the slides and make microphotographs. I was able also to see the naked-eye specimens themselves during a visit I paid to the museum.

These specimens, although now about 140 years old, are in perfect preservation, and it is still more remarkable that the microscopical sections, cut in celloidin and stained with logwood and eosin, show even the finer details in a recognisable manner.

The special fact that emerges is that all the sexual glands are testes in Hunter's cases, that adjacent structures are epididymis, and that in none of the sexual glands are ova present. The characteristic testicular tissue is in the form of tubuli seminiferi, and in only one are spermatozoa present. They thus show the condition usually found in undescended testes in man and in the testis normally before descent is complete. I add a report of Hunter's specimens, and the structures are illustrated in the figures given.

The examination of the slides prepared from John Hunter's free-martin specimens gave the following results:—

Slide 678, testes with tubuli seminiferi; 682, epididymis; 686, epididymis and testes; 688, epididymis; 689, testes and hydatid testis (Müllerian); 690, testes; 691, testes and hydatid testis; 692, testes (Arbuthnot); 693, testes (Wright); 694, testes, scanty elements. In 691 the Müllerian hydatid is well shown (*vide* figs. 3 and 4, Pl. II.).

It seems to me, therefore, fully established that the free-martin, when the co-twin is a potent male, is a sterile male, and not a sterile female, *i.e.* they are identical male twins except in their genital tract and secondary sexual characters.

The following table shows the main cases (thirty in number) I have been able to collect.

Author.	Conditions of Organs.	Remarks.
John Hunter (1786).	(1) *Arbuthnot's Free-Martin.* Vagina, upper part narrow; testes; two cornua; vasa defer., vesiculæ seminales.	
	(2) *Wright's Case.* Vagina with blind end; two horns; testes really, external parts like cow; clitoris.	"More the appearance of the ox or spayed heifer" (*op. cit.*, p. 53).
	(3) *Well's Case.* Teats and udder small; vagina as in (1); vagina and uterus not pervious; testes; vasa deferentia; segments of vesiculæ seminales.	More like a heifer; no microscopic examination made of the tissues.
Numan (1843).	Describes nine cases: six like Hunter's and three somewhat different.	
Spiegelberg (1861).	External genitals and urinogenital sinus as in cow; rudimentary vagina and uterus; parts of seminales and vasa; parts of W. bodies; testes rudimentary (microscopical examination).	Potent bull twin and free-martin calves slaughtered one day after birth; microscopical examination of structures.
Rueff.	Three cases: two had testes; no vesiculæ seminales; external genitals female; vagina(?) blind in all three. *Rep. d. Thierheilk. von Hering*, 12 Jahrg. 1851, p. 103.	
Hering.	Two cases: data only in one. No genitals apart from vagina. ("Ausser der Scheide nichts von Genitalien vorhanden gewesen und von Grunde jener, blos eine dünne Falte des Bauchfells nach beiden Seiten hin abgegangen sein.")	
Anonymous.	In Fuchs' *Thierarztl Zeitung*, Jahrg. iii., note given of five female calves from twins with defective uterus. In another, note is made by Fuchs of a preparation at the Carlsruher Vet. School, of case with both male and female genitals.	
Scarpa.	One year old animal with external female genitals and internal male.	Presence of twin not stated in Spiegelberg's summary.
Gurlt.	Conditions like Spiegelberg's; union between testis epididymis and vas more evident.	
Allnatt.	Vagina long cul-de-sac with rudimentary uterus; solid vesiculæ seminales; vas ended in prostate.	Allnatt states that he was informed of cases where sexual desire was not absent in the free-martin. (There is no reason why it should be absent as a rule, as the testes are usually represented.)

Author.	Conditions of Organs.	Remarks.
Simpson, J. Y. (1844).	Mentions dissection of two adult and one calf free-martin, all like Hunter's cases. Quotes also a case of Allen Thomson's, *Simpson's Obst. Mem.*, i. p. 823.	Simpson does not consider the question of the female calves co-twin with males and fertile as being derived from separate zygotes. This mistake was due to the still current error of holding that sex is not determined, as it really is, on fertilisation, but by subsequent changes in the zygote.
	In *Todd's Cyclopædia*, he quotes a free-martin where he found testicles.	A free-martin is derived along with its potent twin from one zygote.

I have now to explain how the free-martin arises; and in considering this, the cardinal fact must be kept in mind that the potent and sterile twins arise from one zygote, not from two, and that the genitals of each have to be provided from a single zygote, which normally might give rise to one perfect male. Simpson's mistake in his paper was his not recognising that a male and female twin, perfect in development, arise from separate zygotes.

I must now consider—

(1) The General Anatomy of the Genital Tract in the Female Calf.
(2) The Action of Mendelism in producing the Free-Martin condition.
(3) Consideration of the View that the Free-Martin is a transverse Hermaphrodite.
(4) Summary and Literature.

(1) *The General Anatomy of the Genital Tract in the Female Calf.*

This consists of ovaries, Fallopian tubes, cornua, vagina (Müllerian and urinogenital sinus), urethral opening, lateral folds of skin comparable with the human labia majora and minora, and of the glans phalli. The vagina is urinogenital sinus in its lower half and Müllerian in its upper. The urinogenital sinus portion is the lower end of the anterior division of the pars penultima of the primitive gut or entodermal cloaca of other investigators. The lower half of the effective vagina is thus urinogenital sinus, and this must be carefully noted. Developmentally, the sexual glands and urogenital tracts in boves arise as in allied mammals, *i.e.* we have Wolffian bodies, etc., and on these the ovaries or testes develop; subsequent changes in the Wolffian bodies and ducts give us in the female

a persistent Wolffian duct, the canal of Gartner, instead of its uppermost and lowest segments as in most mammals: while the ducts of Müller give the vagina tubes and cornua. The urinogenital sinus, urethra, and bladder arising from the anterior coronal division of the pars penultima of the primitive gut make up the rest of the tract. The ova and spermatozoa arise by reduction from the primitive germ-cells, and these arise not from the germ epithelium but from the primitive germ-cell mass, itself a part of the unreduced zygote in its earliest stage. This view of the origin of the gametes may be termed the zygotic origin of the gametes, or Owen-Weismann law.

In the normal female bovine tract we have not only the potent elements (ovary, cornua, and vagina), but also the epoöphoron with more than the ductus epididymis, viz. the whole Wolffian duct. We have thus in the female the equivalents of the epididymis and vas deferens of the male. These are not functionally active, and histologically are degenerated, but are quite recognisable in the broad ligament.

In the normal bovine male we have not only the potent male organs, but also the hydatid testis (Müllerian duct) and the prostatic utricle, the equivalent of the hymen usually, and a varying amount of degenerated female genital structures.

These potent and non-potent elements of the developed tract mean causal unit-characters in the zygote, of unequal value, usually coupled in the human male and autonomous, Dominant and Recessive in their nature, and it is by their separation or segregation into the soma of the potent male and sterile male (free-martin) that we get the explanation of this anomaly.

(2) *The Action of Mendelism in producing the Free-Martin.*

When a male zygote twins we may get—

(1) Identical male twins.

(2) One perfect male and one sterile male, the free-martin.

In (1) there is an equivalent division of genital and somatic determinants, and identical twins is the result.

How do a perfect male and imperfect male arise?

In the free-martin (2) there are present, as Spiegelberg shows, urinogenital sinus, rudimentary vesiculæ seminales, very rudimentary vagina and knob of uterus; testes and Wolffian bodies, both imperfectly developed.

Now, as already said, we have to account for the genital organs in the potent bull and free-martin from one zygote—a male zygote. Had this male zygote developed a single bull, it would have had potent male organs

(testes and phallus) and non-potent female ones, viz. Müllerian hydatid attached to the testis, and prostatic utricle, the analogue of the hymen, with a varying amount of the rest of the female Müllerian tract. When the twinning of a male zygote in black cattle takes place, this potent and non-potent complex of the genital organs may be divided so that the potent part goes to the potent bull-calf, the non-potent to the free-martin. This being the general scheme of the division, we must now account as exactly as possible for the various parts in the genital tract of the free-martin in Spiegelberg's case, and these may be grouped as follows for explanation:—(*a*) Testes; (*b*) Urinogenital sinus and external genitals; (*c*) Rudimentary vagina; (*d*) Vesiculæ seminales, vasa deferentia, and Wolffian bodies.

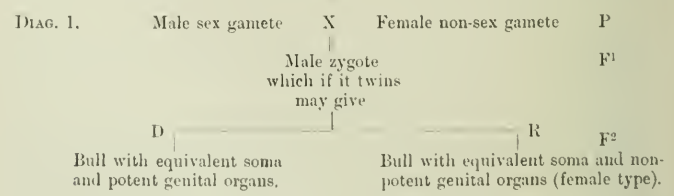
(*a*) *Testes.*—The male zygote gave, of course, sperm-germ cells in the sperm-epithelium of its Wolffian ridges, and the future testes became divided between the twins. This division took place most probably after the primitive sperm-cell mass had formed. The testes are undescended.

(*b*) *Urinogenital sinus and external genituls.*—The former is probably derived from the somatic division of the twins. The labia and glans are non-potent elements.

(*c*) *Rudimentary vagina and uterus.*—These are undoubtedly derived from the Müllerian hydatid and prostatic utricle (Pl. II. fig. 4).

(*d*) *Vesiculæ seminales, vasa deferentia, and Wolffian bodies.* The first two arise from the Wolffian ducts which are present and derived from the non-potent elements; and the Wolffian bodies are given in somatic division.

Thus the free-martin with a potent bull twin is the result of a division of a male zygote, so that the somatic determinants are equally divided, the genital determinants unequally divided, the potent going to the one twin, the potent bull, the non-potent genital determinants to the free-martin. The potent organs are dominant, the non-potent recessive, and the Mendelian scheme may be figured as follows (diag. 1):—

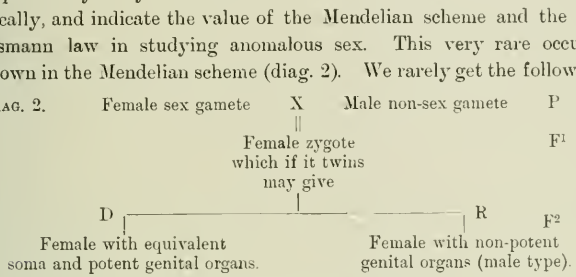


In three of Numan's cases the sterile twin had testes and a rudimentary preputial sheath. This is a rare free-martin where the Müllerian elements remain in the potent bull, but the preputial element of the external genitals

is thrown into this variety of free-martin. There are thus two varieties of bull free-martin, viz. John Hunter's free-martin (so-called heifer free-martin) and Numan's free-martin (steer free-martin).

Before I knew of Numan's paper, I saw that there should be also in Mendel's scheme a free-martin with a potent female.* I could admit this as a possibility not yet demonstrated, but Numan's cases show its existence clinically, and indicate the value of the Mendelian scheme and the Owen-Weismann law in studying anomalous sex. This very rare occurrence is shown in the Mendelian scheme (diag. 2). We rarely get the following:—

Diag. 2.



One point not yet determined so far is as follows:—

While the vaginal and uterine rudiments in the free-martin are undoubtedly derived from the normal non-potent elements of the normal male segregated as a recessive element, as in F^2 of Mendel's crossing pea-experiments, I am in doubt yet as to whether urinogenital sinus and the external genitals are derived from these or from a division of the soma.

(3) *Consideration of the View that the Free-Martin is a Hermaphrodite.*

I may remark here that the non-potent elements in the human genital tract vary in amount. Normally in the male we have only the prostatic utricle and hydatid testis (Müllerian), but occasionally we have the non-potent organs more extensive, producing a uterus and vagina in a male, as in Shepherd's case. These are described as tubular or transverse hermaphrodites. They are not hermaphrodites at all, as no organism is hermaphrodite if the sexual glands are similar. Such anomalies are due to an excessive amount of the non-potent elements; and as these are of the opposite sex type, they give rise to the idea of hermaphroditism. Free-martins thus are not hermaphrodite, and the term pseudo-hermaphrodite means nothing.

(4) *Summary and Literature.*

General Conclusions.—Nature can thus, in a very simple and effective way, sterilise an organism. The zygote, male or female, is, for this purpose,

* As yet there is no actual anatomical demonstration, but Numan gives one of these.

unequally divided by twinning (or otherwise in single cases), so that only recessive or non-potent genital determinants are allotted to the one twin, and in this way sterility is absolutely secured.

On one point we have as yet no information, viz. as to whether or not the potent bull co-twin with the free-martin has its prostatic utricle normal. Theoretically it seems to me this may be defective or wanting, but here actual dissection is necessary.

The free-martin is, according to Mendelian phraseology, a pure or extracted recessive *qua* its genital determinants, and the potent twin a pure or extracted dominant, both of F^2 in the Mendelian scheme. Occasionally, but very rarely, as in three of Numan's cases, the recessive element is less complete.

The potent bull alone can have offspring, and some of its males must breed true to the dominant genital determinants, in certain cases at any rate, as Mendel's scheme demands and theory indicates. This will introduce a variation, *i.e.* we may get a bull not possessing a hydatid testis, and thus varying from the normal bull, and we get here one factor in the mechanism of variation; but I defer the consideration of this.*

According to J. A. H. Murray, the origin of the term "free-martin" is unknown. In Holland they are termed *Kuven*, and in Brabant, *Bouquetin* in France, *Taur*; in ancient Rome, *Taura* was the term (Simpson and Spiegelberg). According to Simpson and others, the Romans did not seem to have been aware of the association of the "Taura" with twinning.

I have to thank Professor J. Arthur Thomson for valuable references, and Mr Henderson for one undoubted specimen.

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* Observations are still needed on such specimens. We need to know the exact condition of the organs in the potent animal, and this could best be done if such twin calves or specimens obtained in the cornua of slaughtered animals were examined as to details. Free-martins are not uncommon, but in Hunter's days they were obtained from the farm and a history given; now, in the large sales where animals are bought, the history can seldom be obtained.

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EXPLANATION OF PLATES.

Plate I.

Genital organs in full-time free-martin calf (Spiegelberg) ($\frac{1}{1}$).

a, urinogenital sinus laid open from the front and turned back: a sound is passed into the Müllerian non-potent element with the knob ending at *c*, the uterus; *d d*, vesiculæ seminales; *e e f*, vasa deferentia; *g g*, imperfect testes; *h h*, Wolffian bodies. In John Hunter's free-martin cases, Owen suggested that in what Hunter figured as double and differing sexual glands, one might be Wolffian body (*vide* Palmer's edition of Hunter, with Owen's notes).

Plate II.

Fig. 1. One of Hunter's free-martin cases. It has the head and horns of the ox or spayed animal, but the fore and hind quarters of a bull. Rudimentary nipples are present.

Fig. 2. Sexual gland in Hunter's free-martin; tubuli seminiferi, slide 678.

Fig. 3. Sexual gland in Hunter's free-martin; epididymis in upper left angle, with Müllerian epithelium and glands below (hydatid testis, slide 691).

Fig. 4. Testis in scrotum of newly-born human fœtus. Testis is below; above, on right side, is seen the hydatid testis unattached and then epididymis attached To left of epididymis lies accessory suprarenal body.

(*Issued separately February* 11, 1910.)

h
h
g
g
f
e
e
d
d
c
b
a

OBSERVATIONS ON EPIDEMIC CEREBRO-SPINAL MENINGITIS.[1]

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(Plates XLVII. and XLVIII.)

The following observations on epidemic cerebro-spinal meningitis are based on a study of the material from over fifty cases of the disease which occurred in Edinburgh and neighbourhood in the winter and spring months of 1907. In a previous communication (1907 [4]), the general pathology and bacteriology of the disease have been dealt with. In this paper attention is limited to (*a*) observations on the staining reactions of the meningococcus; (*b*) the presence of leptothrix forms in the cerebro-spinal fluid in acute cases of meningitis; (*c*) some experimental results in meningococcal infection.

The Staining Reactions of the Meningococcus.

Since Jaeger's original communication (1895 [1]), fierce controversy has arisen with reference to the reaction of the meningococcus towards Gram's strain. A study of the literature on the subject leaves one in little doubt that much of the difficulty has arisen from the confusion of the meningococcus with other diplococci which have no etiological relationship with the disease. The tendency at present is undoubtedly to consider that the entire difficulty may be satisfactorily explained in this way. From my own experience, however, I am thoroughly convinced that at certain times certain strains of undoubted meningococci do show a variable reaction towards Gram's stain, giving at one time a Gram-negative, at another a Gram-positive reaction. That such a reaction is uncommon one may freely admit. In my own series of cases I was able to demonstrate meningococci forty-three times in the cerebro-spinal fluid during life, or in the meninges at the post-mortem examination. In thirty-five of these cases I obtained

[1] Extracts from a thesis for the degree of M.D. of the University of Edinburgh, presented April 1907. Towards the expenses of the investigation a grant was received from the Moray Fund, Edinburgh University. [Received for publication March 12, 1908.]

cultures of the organism, but in only three of the strains did I observe this Gram-variable reaction.

As Gram's stain is applied by different observers in a variety of ways it is necessary to state the precise technique employed in this investigation. Thin films were made and dried at a gentle heat; freshly prepared Gram's gentian-violet solution was applied for four minutes. The solution employed consisted of 1 part of a saturated alcoholic solution of gentian-violet and 9 parts of aniline-oil water. The films were then washed in water and Gram's iodine solution applied for one minute. The films were again washed in water and decolorised in absolute alcohol for at least two minutes. As a counter-stain a 0·2 per cent. watery solution of saffranin was applied for twenty seconds.

I may say at once that I have never observed a Gram-positive reaction in meningococci fresh from cerebro-spinal fluid stained as above, though on more than one occasion I have seen cocci resisting decolorisation with absolute alcohol for one minute. The three strains of meningococci which exhibited the Gram-positive reaction in culture were obtained from the cerebro-spinal fluid during life in typical acute cases of the epidemic form of the disease.

The first case has been already published (1907 [3]). Clinically and at the post-mortem it showed the ordinary characters of acute cerebro-spinal meningitis.

Bacteriology.—The cerebro-spinal fluid obtained during life was brownish and turbid. Large numbers of typical meningococci were present. There were fairly numerous intracellular forms, but the majority were free—between the cells. Practically all were arranged in pairs, and typically flattened on their opposed surfaces. A few looked slightly elongated. Very large numbers of involution forms were present. Variation in size and in intensity of staining was marked. Many large swollen pale coccal forms were observed; some were slightly oval and showed bipolar staining. In some the rim only of the organism was stained at all well; in others the staining was more intense at one side of the coccus, thus giving a signet-ring appearance. The organisms were definitely Gram-negative. A copious growth of the organism was obtained on various media in forty-eight hours at body temperature. In one of the tubes a few colonies developed, apparently from aerial contamination, but in the rest of the tubes only meningococci developed. The colonies, however, differed slightly in size and appearance. Some were very minute, clear, and transparent, and rather resembled pneumococcal colonies. They did not increase much in size, but became slightly more granular. Others were larger from the first, but films from both colonies showed the same organism; and subcultures from the larger colonies gave, in twenty-four hours, colonies indistinguishable from the smaller ones. Films from the original cultures showed the usual appearance of meningococci with great numbers of involution forms. Tetrads were common; a few were rather elongated, most were typically flattened on their opposed surfaces. The great majority were Gram-negative; a few, however, both of the elongated and flattened forms, were Gram-positive. The obvious explanation was that two organisms were present—one Gram-positive, the other Gram-negative, and this view was supported by the appearance of the growth in one subculture of the organism in blood agar. In this case the colonies strongly suggested pneumococci, and films showed some Gram-positive diplococci, some of which were distinctly elongated. Subsequent subcultures, however, on serum agar and blood agar showed only the typical flattened diplococci. A subculture in

human serum showed no development of regular capsules, such as one might expect in pneumococci; only swollen involution forms of meningococci were found. In subsequent cultures the colonies became slightly more granular and opaque. In twenty-four hour growths films stained by Gram's method showed both Gram-negative and Gram-positive forms, but the former ultimately disappeared. A subculture, fifth generation from the original culture, was made on serum agar. In thirty-six hours a fair number of colonies had developed; films showed a pure culture of Gram-positive diplococci. A few drops of sterile bouillon were added to the water of condensation in the tube and a thin emulsion prepared by rubbing down the colonies in the fluid. Seven minims of the emulsion were inoculated into the peritoneum of a mouse. The animal suffered no inconvenience from the injection. On the following day it did not look well, but it took its food and ran about the cage. Forty-eight hours after injection the animal was still active enough, but its coat looked unhealthy. It was then killed by chloroform and a post-mortem at once performed. At first sight there was no evidence of peritonitis; there was no excess of fluid and no solid exudate. Films from the peritoneum, however, showed distinct cellular reaction. Stained by Leishman's method, a fair number of diplococci were present, nearly all intracellular. They were specially situated within the large mononuclear cells, which also showed active phagocytosis towards the polymorphonuclear cells, and occasionally towards red blood corpuscles. Many of the diplococci in the phagocytic cells could be seen still present in the ingested polymorphs. The ingested cells showed all stages of degeneration. Many of the mononuclear cells were vacuolated. Most of the diplococci were typically flattened on their opposed surfaces; many stained perfectly well, others were obviously degenerating. Some of the organisms were Gram-negative; these may have been involution forms, the majority were Gram-positive. Cultures from the heart blood remained sterile, but cultivations from the peritoneum gave a pure growth of the original diplococcus. The organism was kept alive for some time. Most of the organisms remained Gram-positive, but occasionally a few Gram-negative forms were noticed even in young twenty-four to thirty-six hour cultures. At no time was it possible to obtain a culture of this organism in gelatin at room temperature.

I regret that I cannot give an account of the fermentation properties of this organism towards the various sugars. Such observations were attempted, but were not satisfactory or conclusive. Many might justifiably refuse to accept the above evidence as proving that we were dealing throughout with a true meningococcus. In the light of a further experience of similar strains, however, I am convinced that only a single organism was present, and that it corresponded with a Gram-positive variety of an undoubted meningococcus such as has been described by Vansteenberghe (1906 [6]), and others.

The *second* strain in which this Gram-variable reaction was noticed was derived from a rather less acute case of the epidemic variety, which lasted twenty-five days. The clinical diagnosis was confirmed by lumbar puncture during life and by a complete post-mortem examination.

The organism from the cerebro-spinal fluid was a typical meningococcus; uniformly and constantly Gram-negative in films. A pure culture on serum agar was obtained from the cerebro-spinal fluid during life on two different occasions. To naked-eye examination the colonies differed in no way from ordinary meningococcal growths. No growth could be obtained in gelatin at room temperature. In films from the original culture, however, stained by Gram's method, both Gram-positive and Gram-negative forms were present.

This was observed as early as twenty-four hours after inoculation. No elongated forms were noted. The flattened diplococcal character of the organism was constant. Tetrad forms and short chains of three and four elements were also seen. The majority of the cocci were Gram-positive, some were Gram-negative. In some instances, however, one element in the diplococcus was Gram-positive, the other being Gram-negative. This appearance, it must be remembered, was seen in young cultures of from twenty-four to thirty-six hours' growth. A somewhat similar appearance may be sometimes seen in old cultures of various cocci, but I cannot recall having seen this appearance before in adjacent cocci of presumably the same age. Occasionally also in the middle of a short chain, practically all the members of which were Gram-positive, a single Gram-negative coccus was observed.

A thirty-six hours' growth of this organism on serum agar was introduced into the peritoneal cavity of a mouse as before, and exactly the same result was obtained as in the first experiment. The animal was killed after forty-eight hours. There was perhaps slightly more cellular reaction than in the previous case, but the histological appearance of the films was just as before (Plate XLVII. Fig. 1). There were practically no free diplococci present; all were ingested by either polymorphs or large mononuclear cells. The organisms were identical, morphologically, with true meningococci; many were degenerating and showed an acidophil reaction. Stained by Gram's method, most of the cocci were Gram-positive, a few were Gram-negative. A culture from the peritoneum on serum agar gave a pure but scanty growth of an organism, showing the ordinary naked-eye appearance of meningococcal colonies. The organism showed very feeble growth, however, and died out after the second subculture. Films stained by Gram's method showed an appearance exactly similar to that seen in the culture used for inoculation.

The *third* strain of this Gram-variable meningococcus was derived from a very acute fatal case of the epidemic form of the disease. The total duration of the illness was about fifty-seven hours. The diagnosis was confirmed by lumbar puncture during life and by a complete post-mortem examination. In the cerebro-spinal fluid numerous meningococci were present, both free and intracellular forms being noted. The organisms from the fresh fluid were typically Gram-negative. A copious pure growth was obtained on serum agar. In all its cultural characters it corresponded to a true meningococcus. On examining films, however, from the first subculture some Gram-positive cocci were fairly numerous. The appearances were exactly as described in the cultures of the second strain: one half of the diplococcus being Gram-positive, the other Gram-negative. Occasionally also in Gram-positive short chains, single Gram-negative cocci were seen (Plate XLVII. Fig. 4).

A mouse was given an injection (intraperitoneal) of 5 minims of an emulsion of a twenty-four hours' culture of this organism on serum agar. Films from the culture presented the appearances described above. The mouse was found dead next morning about twenty hours after the inoculation. Post-mortem, there was found early sero-fibrinous peritonitis, and the other organs showed acute toxic change. Films from the peritoneal exudate showed enormous numbers of meningococci, with numerous involution forms. There was rather less cellular reaction than in the two previous cases, and very little evidence of phagocytosis. The most important difference was that films stained by Gram's method showed the organisms to be uniformly and completely Gram-negative (Plate XLVII. Fig. 3). Pure cultures of meningococci were obtained from the peritoneal cavity and heart blood, and in these cultures and in subsequent subcultures the organisms retained their Gram-negative character. No Gram-positive elements appeared.

I do not see how those last results can possibly be explained otherwise than on the hypothesis that we were dealing with a

Gram-variable variety of the meningococcus which was typically Gram-negative in the tissues, but in certain cultures showed Gram-positive characters. The organism was evidently much more virulent than in either of the two previous cases.

It would be futile and quite unnecessary to try to awaken at this date a controversy on this much disputed point. Jaeger (1901 [2]), gives a complete account of the work of various observers, including that of Mayer, Pfaundler, and Fränkel, who insist on this Gram-variable reaction of the meningococcus; and, as has been stated, Vansteenberghe, (1906 [6]), has come to the same conclusion. It is not surprising that an organism so variable in its characters as the meningococcus is known to be should show a varying reaction to a stain which, however valuable in the differentiation of certain organisms, does not give constant results in a not inconsiderable group of bacteria. The matter is perhaps after all not of supreme importance, but it must be insisted on that special tenacity of Gram's stain in the case of some particular diplococcus from a case of acute meningitis is not sufficient to definitely exclude the organism from the true meningococcus group. The reaction of the organism in the living tissues, as tested by animal experiment, must be carefully studied. Useful as the various fermentation tests for the meningococcus undoubtedly are, it is questionable if they should constitute the sole and final court of appeal in doubtful cases.

The Occurrence of Leptothrix Forms in the Cerebro-Spinal Fluid in Cases of Acute Meningitis.

In February 1907 I was asked by Dr. John Thomson to examine the cerebro-spinal fluid in a case of acute meningitis. In his experience the case differed in no way, clinically, from the epidemic form of the disease which was then prevalent. On lumbar puncture $2\frac{1}{2}$ drms. of almost clear cerebro-spinal fluid were obtained. Films showed numerous polymorphonuclear leucocytes and a few elongated diplococci, somewhat suggesting pneumococci; the organisms were definitely Gram-negative, and no intracellular forms were observed. The most striking feature in the films, however, was the presence of numerous long thin irregularly and faintly staining bacilli; some straight, many slightly curved. These organisms were also Gram-negative. Cultivations were attempted on serum agar and blood agar, but no growth was obtained. I was at first inclined to attribute these bacillary forms to a contamination, but subsequent examination convinced me that even the diplococci were merely stages in the development of the longer thread forms, and my experience in the case next to be described confirmed my opinion that we were dealing with a leptothrix invasion of the cerebro-spinal fluid. No meningococci or pneumococci were demonstrated in this case. It is possible, of course, that either of these organisms may have been originally present but had died out. The case terminated fatally in about eighteen days. Unfortunately no *sectio* could be obtained.

A second case of the same description was admitted to the Royal Hospital for Sick Children, Edinburgh, on 9th February 1907. The patient was discharged to the City Fever Hospital, and died there after a total illness of eight days. Clinically, in the opinion of Dr. John Thomson it could be in no way distinguished from the other cases of acute cerebro-spinal meningitis.

On 9th February 4 c.c. of slightly turbid fluid were obtained by lumbar puncture. Films from the deposit showed numerous polymorphonuclear leucocytes and some elongated diplococci, suggesting the appearances of the shorter forms seen in the previous case. In addition, some longer bacillary forms, irregularly staining and definitely Gram-negative, were present. These also closely resembled the shorter of the thread-like forms seen in the previous case. The organisms were entirely extracellular, and were not so numerous as in the first case. No diplococci resembling either pneumococci or meningococci were discovered. Again suspicious of a contamination, I obtained another specimen of cerebro-spinal fluid three days later. The appearances of the films were practically similar to those seen from the first specimen. Cultures were made from the first specimen on blood agar and on Löffler's serum smeared with sterile blood. No growth appeared on the blood agar for three days. At the end of that time numerous colonies appeared of what seemed to be pneumococci. The colonies were small, round, discrete, and almost transparent, but on making films long thin bacilli, in some cases slightly curved, were found. The bacilli in their morphology and staining characters corresponded entirely with the bacilli in the cerebro-spinal fluid. On the next day films showed a tangled mass of sinuous filaments. In some films stained by Gram's method minute points could be seen in the filaments retaining the stain, but the filaments themselves were rapidly and completely decolorised. No true branching was observed. The organism corresponded to a leptothrix form. Among the filaments some small oval highly refractile bodies were seen in the older cultures, and in addition some minute diplococci very distinctly Gram-positive were noted. About six days after the tube was inoculated a fine white powdery appearance was seen on the surface of the medium, a little suggestive of the powdery efflorescence seen sometimes in cultures of streptothrix forms. Later a grey-white wrinkled film developed over the water of condensation and the sloped surface of the medium. No difficulty was experienced in obtaining subcultures on ordinary media, but the bacilli in these subcultures appeared as shorter plumper rods, and I am not able to say with certainty that these were not due to secondary aerial contamination, the original leptothrix being crowded out. These later cultures were what were used for the inoculations described below, and this must be remembered in deducing absence of pathogenic property in the leptothrix.

The diplococci mentioned above were isolated in subcultures. The same organism appeared on the Löffler's serum media. They were constantly and strongly Gram-positive. In subsequent cultures they assumed a staphylococcal character. They were rather smaller than *Diplococcus crassus*, but otherwise they corresponded with that organism. A post-mortem (limited to the central nervous system) was obtained in this case.

Spinal cord.—On opening the dura there was found distinct hyperæmia of the pia arachnoid, with some fine recently formed adhesions between dura and pia. There was no distinct recent exudate visible to the naked eye.

Brain.—There was well-marked fibrino-purulent leptomeningitis, best marked over the sides and vertex of the brain anteriorly, but also present on the under surface of the temporal lobes. Otherwise the base of the brain was free from exudate. The exudate was yellow-white in colour, and occurred in patches with sharply defined outlines. These patches completely covered areas on the cerebral surface up to an extent of 1½ inch in diameter. Films from the exudate showed a few bacilli similar to those seen in the cerebro-spinal fluid during life, and a few diplococci resembling those which developed in the cultures. Cultures, however, on various media remained sterile. No evidence was obtained of any special pathogenic property of the leptothrix. A mouse inoculated (intraperitoneally) with 7 minims of the original cerebro-spinal fluid suffered no inconvenience, and remained perfectly well. A thick emulsion was prepared in broth from a forty-eight hours' surface

growth of the leptothrix in a 20 c.c. flask of bouillon. The following doses of the emulsion were given respectively to a mouse, a young guinea-pig, and a young rabbit, namely, 5 minims, 10 minims, and 14 minims. The mouse and guinea-pig were inoculated intraperitoneally, the rabbit intravenously. None of the animals suffered the slightest inconvenience, and remained perfectly well.

I have since seen three other cases of this leptothrix invasion of the cerebro-spinal fluid. The organisms in these latter cases corresponded exactly to those described in the first two cases. In one case no other organism was found; in the second a true meningococcus was present with the leptothrix, and in the third a coccus was present which may have been *Diplococcus crassus*. Its exact nature was not determined. In each of the three cases there was very active phagocytosis of the bacilli by the polymorphonuclear leucocytes. In none of them was any growth of the leptothrix obtained, though repeated attempts were made on various media. In one only of the cases was there an opportunity of examining the brain post-mortem, though all terminated fatally. The exudate in this case was much less abundant than in the previous case which came to post-mortem examination, but it had the same general characters and distribution.

As regards the general significance of this leptothrix invasion, the evidence at present hardly warrants a definite opinion. There is nothing to indicate that it is the causal organism. The most natural explanation is that it has found its way secondarily into the spinal canal, and its association with organisms other than the meningococcus favours this view. It may be that the cases are primarily meningococcal, the meningococci being either extremely scanty or having died out altogether. On the other hand, it would appear that the infection has at least been modified by the presence of the bacilli, as the exudate in the two cases I have seen has been very definitely much more fibrinous than in meningococcal meningitis, and its distribution has not been quite that seen in the epidemic cases. Even if it be regarded as a purely secondary infection, however, it is interesting as suggesting a possible route of invasion of the real causal organism, such leptothrix forms being most usually found in infections about the mouth, pharynx, or lungs. It is quite possible that in other cases of serious disease in the central nervous system, bacilli, secondarily invading the spinal canal, may be mistaken for the primary cause of the disease.

Some Experimental Observations on the Meningococcus.

The animals used were specially mice and monkeys, but a few experiments were also made on guinea-pigs and one on a goat. Of the smaller animals the mouse is, in my experience, by far the most suitable animal for experimental work. The results, however, vary to

a very considerable extent: this can be readily understood when we remember the great variation in virulence which the meningococcus may exhibit. The experiment quoted in the case of the third strain, which showed the Gram-variable reaction, is typical of what is seen when a virulent variety is employed. The rate at which the virulence of the organism becomes diminished varies greatly. In the case of some of the older cultures even small mice resisted enormous doses. Though I have only made a few observations on cultures long removed from the body, in one case at least the organism appeared to be quite as virulent to mice nine weeks later as in the first sub-culture. Fairly constant results followed the intraperitoneal injection of from 5 to 7 minims of the cerebro-spinal fluid fresh from the body of acute epidemic cases, the animals almost invariably dying in from twelve to thirty hours. Only in two cases where fresh cerebro-spinal fluid was used were negative results obtained. In these two cases two mice were inoculated with 5 and 7 minims respectively of fluid obtained by lumbar puncture in an acute adult case. Organisms were fairly numerous in the films. Neither animal suffered any inconvenience, and both were alive and well one month later. On the other hand, in another case where the organisms were extremely scanty in the original fluid from an acute case a fatal result was obtained in about thirty hours by the injection (intraperitoneally) of 7 minims of the fluid. There was apparently distinct multiplication of the organisms in this case, and though no growth could be obtained from the original fluid a fairly copious growth was obtained from the peritoneal exudate and also from the heart blood. It is therefore advisable, in cases where the organisms are very scanty in the fluid, and where it is important that a culture should be obtained, that an intraperitoneal injection of the fluid should be made in a mouse; in this way the organism may be secondarily recovered.

In contrast to this case, however, it must be admitted that in one case, where the organisms were very numerous in the original fluid and no difficulty was experienced in obtaining a fairly copious growth, only three colonies were obtained from the peritoneum of a mouse inoculated with the fresh fluid and dying in twenty-six hours. Films from the peritoneum in this case showed very large numbers of the organisms, many staining perfectly well. This only illustrates, however, the well-established point that only a very minute percentage of the cocci in the tissues are viable.

In a very considerable proportion of the cases of infected mice the organism was recovered from the heart blood as well as from the peritoneum. It is a point of some importance, in examining infected mice, to note that to the naked eye there may be very little reaction in the peritoneum.

I have made no special study of the relative proportion of the inflammatory cells in the peritoneal exudate at different periods. As

far as I have observed, however, the appearances closely resemble those seen in peritonitis artificially induced by such an organism as *Bacillus coli communis*. In the milder cases that are on the way to recovery active phagocytosis on the part of the large mononuclear cells is a special feature. In mice receiving very large doses of culture there may be very little cellular reaction, the animals dying of a pure toxæmia.

Guinea-pigs even when young have, in my experience, not been so satisfactory for experimental work.

In one case, for instance, a young guinea-pig was given (intraperitoneally) 12 minims of an emulsion of a twenty-four hours' growth of a meningococcus from an acute case. Five minims of this same emulsion killed a mouse in less than thirty hours, but the guinea-pig lived for sixteen days, and at the *sectio* I was unable to recover the organism.

One experiment was performed on a young goat with negative result. The virus employed consisted of 30 minims of turbid cerebro-spinal fluid containing numerous meningococci, from an acute epidemic case in a child. A subdural spinal inoculation was made in the region of the upper lumbar vertebræ. Some difficulty was experienced in introducing the needle, owing to the overlapping of the laminæ, but ultimately a few drops of clear cerebro-spinal fluid escaped. The injection was then made; the animal did not appear to suffer the slightest inconvenience either at the time or subsequently. Five minims of the same fluid gave a rapidly fatal result in a mouse.

It is interesting to note the varying results which have been obtained by different observers in the case of goats. Heubner (1896 [7]), obtained a positive result, as did also Councilman, Mallory and Wright, (1898 [8]). On the other hand, Bettencourt and França failed (1904 [9]), and though Albrecht and Ghon (1901 [10]) had a fatal result with convulsions, they failed to recover the organism, or indeed to demonstrate any pathological change in the brain or cord.

Experimental Work on Monkeys.

Very little work has been done in this direction till quite recently. In 1904 Bettencourt and França attempted to reproduce the disease in apes, but failed. In 1906 Flexner recorded a series of successful attempts through intraspinal injections of cultures of the meningococcus. Later, in 1906, von Lingelsheim and Leuchs published the results of two successful experiments, and Flexner, (1907 [3]), gave a full account of his work up to that date. Flexner was able to reproduce all the essential features of the disease. I do not propose to make any reference to the results he obtained in inducing immunity towards the artificially produced disease, as I have done no work in this direction myself.

In March and April 1907 I performed seven experiments on monkeys, and give below an abstract of the result in each case. The doses of the cultures employed by Flexner are admittedly very large. In six of my experiments I used small doses of cerebro-spinal fluid from acute and subacute cases of the epidemic form of this disease, so

that the number of organisms introduced has been considerably less than Flexner employed. The action, however, may possibly have been intensified by the presence of aggressins in the cerebro-spinal fluid. In all my cases also I gave a single injection only in each case.

Experiment 1.—*March* 1.—A fair-sized monkey (*Rhesus*) was inoculated with a sixty hours' culture (second generation) of a meningococcus obtained post-mortem from an acute case. The colonies on one serum agar tube were rubbed down in sterile broth, and 20 minims inoculated subdurally in the lumbar region. The animal was etherised. On recovering from the anæsthetic it showed no bad effects. There were never any meningeal symptoms; the animal died about three months later considerably emaciated, but at the post-mortem examination extensive tuberculosis was found. There was, however, no evidence of meningitis in brain or cord. This negative result was probably due to the age of the culture.

Experiment 2.—*March* 17.—A monkey (*Rhesus*) was given a subdural injection of 15 minims of the centrifugalised deposit from cerebro-spinal fluid from an acute case of the epidemic variety. The organisms in the fluid were very scanty. The animal was etherised and the parietal bone exposed; a minute trephine opening was then made through the skull and a fine needle introduced just below the dura. The fluid was slowly introduced and the wound closed. The next day the animal looked perfectly well. In the afternoon it had a convulsion lasting for about three minutes. In the evening it was distinctly ill. It showed great weakness of the arms and clonic lateral spasms of the neck with a general condition of hypertonus. There was no rigidity of the limbs. At 11 P.M. lumbar puncture was performed, the fluid was slightly turbid and showed an excess of polymorphs, but no organisms were discovered, and cultures from it remained sterile. The next day there were some slight convulsive attacks, but the animal gradually recovered, and for six weeks remained quite well. It then began to show some emaciation, and ultimately died about fourteen weeks after inoculation.

Post-mortem.—The most striking change was the enormous thickening of the skull which was present: the bone was soft and vascular; the condition had certainly developed since the operation, but there is no evidence that it was a direct result; similar conditions I understand, though hardly as extreme, are not uncommon in monkeys, and depend on improper diet, etc. There was slight but distinct chronic leptomeningitis. The dura was adherent to the pia at the seat of puncture. No organisms could be discovered in films or demonstrated in sections. Sections of the cortex showed slight thickening of the pia arachnoid with dilatation of vessels in the brain itself. The spinal cord showed nothing abnormal either on naked-eye or microscopical examination. In spite of the early symptoms and evident meningitis one cannot regard this as a proved case, as the organisms were never demonstrated. The histological appearances, however, are not unlike what one sees in cases of very chronic posterior basal meningitis. None of the other organs showed any special change.

Experiment 3.—*March* 17.—A small monkey (*Callithrix*) was inoculated with 12 minims of cerebro-spinal fluid from a subacute case of meningococcal meningitis. The organisms were numerous in this case. The same procedure was adopted as in Experiment No. 2, and the fluid injected through the parietal bone. On the evening of the following day the animal was dull and apathetic; it would not move from the corner of the cage; later it became weak and lay on its side. There was apparent blindness; from time to time there were lateral jerking movements of the head, but there were no general convulsions. The next morning it seemed better and was able to sit up, but it

never quite recovered. There was slight rigidity of the back before death, which occurred five days after inoculation.

Abstract of Sectio.—Early leptomeningitis with some small hæmorrhages. There was no evidence of injury to the brain by the injection needle. On the surface of the brain, about ½ inch from the point of inoculation, there was a small superficial area of softening. There was also early spinal meningitis. In the abdomen the only point worthy of special note was extensive hæmorrhage in the gastric mucosa. Films from the surface of the brain at some distance from the point of inoculation showed meningococci, mostly degenerated, however. Several impurities appeared in cultures from the brain, and the meningococcus was not recovered.

Histology.—The lesions in the cerebral meninges were strictly comparable to the changes seen in the natural disease; but there was more perivascular infiltration with lymphocytic cells than is seen in man. The appearances in the spinal meninges also resembled those seen in man, but there was again more infiltration along the lymphatics and more evidence of degenerative change in the nerve cells than is seen in the human cases. Meningococci were demonstrated in sections of the cerebral meninges, but nowhere else. The other internal organs showed acute parenchymatous change.

Experiment 4.—A monkey (*Callithrix*) was inoculated on March 18th with 14 minims of cerebro-spinal fluid from an acute case. The organisms were fairly numerous in the fluid. The animal was etherised and the fluid introduced into the spinal subdural space between the seventh and eighth dorsal vertebræ. The next morning, seventeen hours after injection, the animal was obviously ill. It was dull and subdued and took little notice of movements in its vicinity. Before inoculation it was noted as being wild, ferocious, and vicious. In the course of the morning it developed weakness in its arms, and showed a tendency to tumble forwards. There was at this time no rigidity of the limbs. It gradually became weaker and semi-comatose. There was slight head retraction; there was general rigidity of the spine and tail, but the limbs were flaccid. The coma deepened and the animal died twenty-five and a half hours after inoculation.

Abstract of sectio.—The *spinal cord* showed early diffuse leptomeningitis, best marked in lower part of cord. On opening dura a thin purulent fluid was seen in subdural space. Films of this showed numerous meningococci. *Brain.*—Over vertex there was a faint general turbidity, due to a thin layer of yellow exudate shining through the dura. On slitting the dura the pia arachnoid was found unduly hyperæmic, and there were a few punctiform hæmorrhages scattered over the cortex. There was also exudate at the base of the brain, more advanced than over vertex; films from the exudate over vertex and base also showed numerous meningococci. The organisms were recovered in culture from both brain and cord. The other internal organs showed acute toxic change. There were numerous hæmorrhages up to $\frac{1}{16}$th inch in diameter below the mucous membrane in the stomach. There was also some early intestinal catarrh, with prominence of the lymphoid tissue and slight enlargement of the mesenteric glands; these latter were pale and firm. There was a considerable quantity of recent dark red blood clot in the intestine. These acute secondary manifestations in the alimentary canal appear to be of special importance, for without a previous knowledge of the case they might have been taken as primary lesions indicating a primary intestinal infection.

Abstract of histological observations.—Acute leptomeningitis much more marked than in the previous case, but with the same general features in the meninges and brain. In the cord there was well-marked exudate on the surface and extending along the nerve roots; there was also marked perivascular lymphocytic infiltration into the substance of the cord (Plate XLVII.

Fig. 2), and a collection of mononuclear cells in the central canal which was badly defined owing to an acute inflammatory softening. There were other areas of acute softening in the cord. The exudate over the cerebral cortex was extremely like that seen in man in the acute cases. There was comparatively little fibrin, with many large mononuclear phagocytic cells. A few meningococci, both free and intracellular forms, were demonstrated in the sections.

The results in Experiments 3 and 4 corresponded so completely with Flexner's observations that it was not thought necessary to perform any further experiments of the same nature. In order to demonstrate the effects of infection by other paths, three other monkeys were inoculated. These will be only briefly referred to at present.

Experiment 5.—A monkey (*Callithrix*) was inoculated intravenously with 15 minims of cerebro-spinal fluid from an acute epidemic case. The animal died on the fourth day with symptoms of acute toxæmia. There were no meningitic symptoms.

Post-mortem.—No meningitis; acute general toxæmia. No meningococci were discovered in any of the organs, which showed simply acute parenchymatous change.

Experiment 6.—A small monkey (*Rhesus*) was given an injection of 15 minims of an emulsion of a twenty-four hours' growth of a meningococcus from an acute case. The animal was etherised, and the injection made into the sheath of the sciatic nerve. Though injected slowly, much of the fluid escaped into the surrounding tissues. The object of the experiment was to determine the possibility of the infection of the cord by way of perineural lymphatics. A culture was used, as no fresh fluid was available at the time. I have reason to believe that the organism used was one of low virulence. The animal showed no symptoms of any disease for between three and four weeks, but died rather unexpectedly just over four weeks from the time of injection.

Post-mortem.—There was no apparent cause for death. Cultures from the surface of brain and cord and from the region of the inoculation into the sciatic remained sterile. There was no appearance of meningitis either to the naked eye or on microscopical examination. No meningococci or other organisms were found in the tissues. Locally at the point of inoculation there was chronic inflammatory change in the sciatic itself and in its sheath.

Experiment 7.—In another paper already referred to I have indicated certain points in favour of the view that we sometimes have a primary intestinal infection with the meningococcus. The following experiment was designed to throw light on this point:—

A monkey (*Callithrix*) was etherised, and abdominal section performed. Twenty-five minims of cerebro-spinal fluid from an acute meningococcal case were then injected into the jejunum through a fine hypodermic needle, and the abdominal wound was then closed.

The animal recovered perfectly from the operation, but showed symptoms of general toxæmia. There were no indications of meningitis; there was slight diarrhœa. The monkey refused food, gradually became weaker, and died on the fourth day.

Abstract of sectio.—There was no peritonitis; there was general acute toxæmia, but no meningitis (cerebral or spinal). There was slight recent enlargement of the mesenteric glands near the jejunum. Cultures from the brain, heart-blood, and mesenteric glands unfortunately became contaminated from blood serum added to the agar just before use.

Abstract of histological appearance.—The mesenteric glands were acutely

hyperæmic, and showed marked endothelial proliferation with necrotic change; Gram-negative diplococci, apparently meningococci, were present; many were degenerated and, staining badly, were difficult to define. The subcapsular lymph space was injected with lymphocytes closely aggregated and apparently distorted from pressure. The wall of the small intestine near the site of inoculation showed lymphocytic infiltration in the submucous coat. Much cell débris was present, and it was difficult to say definitely that meningococci were present here. There was no evidence of meningitis in sections of the brain and cord; the other organs showed acute toxic change.

Though no meningitis was produced in this experiment, the fact that the meningococcus can produce a fatal intestinal toxæmia and invade the neighbouring lymph glands appears of importance, in view of the possible primary intestinal route of infection in the naturally acquired disease.

Although nothing very definite can be concluded from the results of the last three experiments, they indicate further possible lines of research in the direction of determining the paths of invasion of the body in ordinary meningococcal infections. It must be confessed that the routes of infection are by no means satisfactorily established so far.

The earlier experiments were kindly performed for me by Dr. J. M. Beattie in the Pathological Department of the University of Edinburgh. The later work was carried out in the Laboratory of the Royal College of Physicians, Edinburgh, and I have to express my sincere indebtedness for the facilities there afforded me.

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DESCRIPTION OF PLATES XLVII. AND XLVIII.

Plate XLVII.

Fig. 1.—Film from peritoneum of mouse infected with the second strain of meningococci, giving a Gram-variable reaction (p. 445). Stained by Leishman's method. (× 1300 diameters.) Showing appearances seen in a mild meningococcal infection. There is active endothelial proliferation with marked phagocytosis.

Fig. 2.—Transverse section of spinal cord of monkey (Experiment 4, p. 452). Leishman's stain. (× 105 diameters.) Showing acute pacchy- and lepto-meningitis, with marked lymphocytic infiltration round the posterior nerve roots, and extending along the vessels into the substance of the cord.

Fig. 3.—Film from peritoneum of mouse infected with a virulent strain of meningococci. Gram's stain ; counter-stain, saffranin. (× 1000 diameters.) This organism was from the third strain showing the Gram-variable reaction (p. 445). The organisms here were uniformly Gram-negative.

Fig. 4.—Film from a 24-hours' culture of the same strain seen in Fig. 3. This was the appearance of the culture that was actually used for inoculation of the mouse referred to in description of Fig. 3. Gram's stain ; counter-stain, saffranin. (× 1500 diameters.) Most of the organisms here are Gram-positive ; some are Gram-negative ; diplococci are seen with one element Gram-positive ; the other Gram-negative. (Note the higher magnification than in Fig. 3.)

The illustrations were drawn to scale by Mr. G. Buchanan, Department of Pathology, Edinburgh University.

Plate XLVIII.

Fig. 5.—Film from a 24-hours' serum-agar culture of a typical meningococcus. (× 1000 diameters.)

Fig. 6.—Film from the cerebro-spinal fluid in the second leptothrix case described on p. 447. Showing bacillary and diplo-bacillary forms. Stained by Leishman's method. (× 1000 diameters.)

Fig. 7.—Film from a 4-days' culture on blood agar, from the cerebro-spinal fluid in the second leptothrix case. Showing the development of long non-branching filamentous forms. Stained with dilute carbol-fuchsin. (× 1000 diameters.)

Fig. 8.—Film from a 24-hours' subculture on serum agar of the diplococcus isolated from the cerebro-spinal fluid in the second leptothrix case. (× 1000 diameters.) This organism shows tetrads, short chains, and a tendency to a staphylococcal arrangement of the elements, which were all strongly Gram-positive. Its nature is uncertain. It resembles *Diplococcus crassus*, but is smaller (compare with Fig. 5).

Fig. 9.—Film from the peritoneum of a mouse injected (intra-peritoneal) with 5 minims of cerebro-spinal fluid from an acute meningococcal meningitis. Showing the result of a virulent meningococcal infection. There is little cellular reaction ; many red blood cells are present, and numerous meningococci with swollen involution forms. Stained by Leishman's method. (× 1100 diameters.)

The photo-micrographs are by Mr. William Watson, Laboratory of the Royal College of Physicians, Edinburgh.

Fig. 1.

Fig 2.

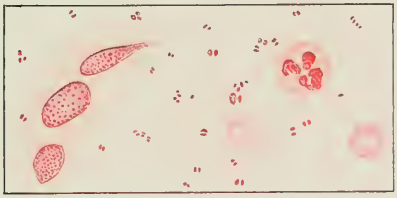

Fig 3.

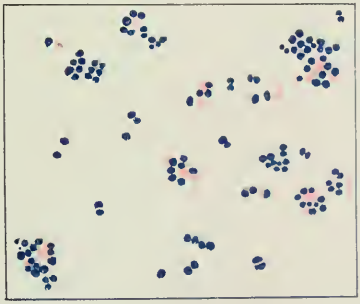

Fig 4

AN EXPERIMENTAL INVESTIGATION OF INFECTIVE SARCOMA OF THE DOG, WITH A CONSIDERATION OF ITS RELATIONSHIP TO CANCER.[1]

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(Plates XLII.–XLVI.)

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In recent years there is probably no morbid process which has been the subject of so widespread investigation and speculation as cancer. It will be conceded by most that little further advance in our knowledge is to be hoped for by clinical or histological investigation alone. The difficulty which has been met with by those who have been engaged in the experimental investigation of this disease has been that, so far, in most cases where portions of tumour growth have been inoculated into animals of the same or different species,

[1] Abstract of a thesis for the degree of M.D. of the University of Edinburgh, and awarded a gold medal, July 1907. Received December 5, 1907.

the result has been negative and further research has been arrested. The investigation, therefore, of infective sarcoma, which is a disease possessing, both clinically and pathologically, so many points of resemblance to sarcoma as it occurs in man, and in which inoculation experiments readily succeed, appeared to me to offer fair prospects of results of value.

I. The Characteristics of the Disease following Natural Infection.

Clinical Features.

The disease known as infective sarcoma is one which occurs in dogs. It is not uncommon, and appears to have a world-wide distribution. In this country, on the continent of Europe, and in the United States of America it is frequently met with. C. G. Seligmann (1906) has found it in the native dogs from the coast villages of the central division of British New Guinea, where, according to the natives, it was endemic before the advent of the white man. It is a contagious venereal disease, the transmission of which occurs during coitus; thereafter there develop on the genital tract of the animal infected soft, fleshy, highly vascular tumours, having, to the naked eye, an appearance similar to a rapidly growing sarcoma, and a histological structure identical with that of an alveolar sarcoma. The remarkable readiness with which this disease may be conveyed by contagion has long been known to dog fanciers, and the investigations of Smith and Washbourn (1898) have demonstrated this point very clearly.

The material used in this investigation was originally obtained from two sources. One was a bull bitch, the property of Dr. Beard of Edinburgh University, the other a wire-haired fox-terrier, which was kindly presented to me by Dr. Bashford, Director of the Imperial Cancer Research Laboratory, London.

Case 1.—In the bull bitch the disease had been contracted from an unknown source, but it was known to have suffered from it for more than a year. The animal was of mature years, and when first seen it was obviously ill. It was languid, cowed, and considerably emaciated. The vulvar orifice protruded, and definite lobulated swellings were visible on either side projecting underneath the skin surface adjacent to the vagina. The vaginal cavity was occupied by confluent tumours of a fleshy consistence, which bled readily when touched. Some of these lay underneath an ulcerating surface, others were felt beneath the vaginal mucosa, and finally the nodules visible from without were found to extend from the vaginal to the skin surface.

Case 2.—This was a wire-haired fox-terrier dog, and had situated underneath its foreskin, growing from the corona of the penis, a cauliflower-like mass of new growth of a soft fleshy consistence. The size of the tumour mass was such as to render protrusion of the organ impossible, except under an anæsthetic. The histological structure of this tumour was similar to that obtained from the former case.

Morbid Anatomy.

The first animal was killed a fortnight after the original examination, and at the post-mortem examination the appearances found during life were confirmed. Confluent tumour masses occupied the vaginal cavity. On section these had a light pink colour and were of a uniform fleshy consistence, and some were seen to be invading the adjacent muscle. The majority possessed a definite capsule and could be enucleated with ease. The tumours were entirely confined to the vaginal region, and no secondary foci could be detected in the regional lymphatic glands or anywhere throughout the body. This state of affairs corresponds with that described as characteristic of this disease. The tumours formed in the course of natural infection usually remain local, and death in an untreated case is brought about either by septic absorption from an ulcerating surface or invasion of neighbouring organs. Secondary foci of tumour growth occasionally occur, and were present in one of my cases where the animal died after successful inoculation.

Histological Structure.

Throughout this investigation saturated corrosive sublimate solution was found to be the best fixative, and for stains the following reagents were used in every case:—

Hæmatoxylin and eosin (H.E.).
Hæmatoxylin and picro-fuchsin (H.Pf.).
Hæmatoxylin and iron alum (H.Ia.).
Hæmatoxylin, iron alum picro-fuchsin (H.Ia.Pf.).
Eosin and methylene blue (E.Mb.).
Pyronin, resorcin, and methyl green (P.R.Mg.).

Microscopical examination of portions of the tumour removed during life from Case 1 showed (Plate XLIV. Fig. 5), in the subcutaneous tissue beneath the vaginal mucosa, a mass of new growth possessing a delicate capillary stroma, forming spaces containing cells which were arranged in an irregular manner. The tumour cells were polyhedral, and virtually uniform in size and structure. They measured from 9 to 11 μ in diameter. The typical cell has a large cell body containing a large nucleus eccentrically placed, and having within it a distinct nucleolus. Many tumour cells can be seen to be in various stages of indirect cell division. Tripolar mitosis as described by Bashford (1905) can occasionally be seen. I have never been able to detect heterotype mitosis in any of the specimens examined.

The tumour cells could be seen to be invading the adjacent fibrous tissue and muscle, and occasionally a portion of this is seen included inside the tumour. At other parts the new growth possesses a distinct fibrous capsule. With E.Mb. the tumour cells stain in a characteristic manner.

The cell body stains a light blue and is finely granular.

The nucleus stains a darker blue colour, and has an open chromatin network. With P.R.Mg. the finer structure of the tumour cell is especially well brought out. The cells are polygonal in shape, the cell membrane is faint in outline, the contained cytoplasm stains a light salmon colour; the nucleus, which is large, stains of a dark slate blue colour, and contains darker granules of a similar shade, in addition to a single true nucleolus which stains a bright rose-red with pyronin. Especially by means of this differential stain there can be made out, throughout the tumour, other cells, some of which are small lymphocytes, others are similar in appearance to large lymphocytes, and finally a third variety is present which is almost identical with the tumour cell, but can be recognised by differential stains to be similar to the cells described by Maximow (1902) as polyblasts.

The three varieties of cells described are seen throughout the tumour. They are few in number, and show all transition forms from lymphocytes to polyblasts and occasionally to plasma cells.

At the border of the tumour towards the vaginal surface (Plate XLIV. Fig. 5) the tumour cells are seen invading the adjacent connective tissue. It would appear to be the case that the formation of additional tumour cells was taking place from the cells of the tissue invaded, but this is difficult to be certain of. Where the tumour is encapsulated the "capsule" can be seen to consist of elongated spindle-shaped cells, which are young fibroblasts, and have a staining reaction similar to the tumour cell. In this region the polyblasts are abundant. From their morbid anatomy and histological structure the tumours which occur in connection with the disease known as infective sarcoma have generally been classified as sarcomata. With that opinion I agree. They possess a structure indistinguishable from a true alveolar sarcoma.

Smith and Washbourn (1898) considered the tumours to be certainly sarcomatous in structure, and that "they did not differ in structure, or malignancy from the sarcomata met with in the human subject."

Wehr (1889) was of the opinion that the tumours were carcinomata. He successfully inoculated the tumours into dogs, and obtained secondary deposits in various internal organs in some cases.

Duplay and Cazin (1894) thought the structure of the tumour resembled inflammatory rather than carcinomatous tissue. They also transmitted the disease to other dogs by subcutaneous inoculation.

Geissler (1895) described the results of his study of this disease to the German Surgical Congress, and gave as his opinion that the growths were carcinomata. In the discussion which followed—

Hansemann stated that he had found such tumours showed a mixture of granulation and sarcomatous-like tissue.

Israel agreed that the preparations exhibited were not carcinomatous.

Powell White (1902) studied the disease in two animals. In his opinion it was a lymphosarcoma.

Sticker (1904) considered it to be a lymphosarcoma.

Bashford, Murray and Cramer (1905), who especially investigated the

histogenesis of the tumour formed after subcutaneous inoculation, were of the opinion that the tumour resembled new growths in its histological features. The transformation of connective-tissue cells into tumour cells at the extending border of the nodule, which they figure as occurring, militated in their opinion, however, against the view that the growth was a true sarcoma. On this ground, and on others to be referred to later, they would prefer to describe the disease as an infective granuloma.

M'Fadyean (1905) says these tumours are not true sarcomata, and have a histology closely resembling granulation tissue.

Beebe and Ewing (1906) express the opinion that the general histological characters of the disease suggest to them the diagnosis of alveolar sarcoma or endothelioma.

II. The Propagation of the Disease by Inoculation.

1. *Inoculation into Animals of the same Species.*

In order to determine the facility with which the tumours could be reproduced in other animals of the same species, portions of the original "natural" tumour were inoculated into the subcutaneous tissue of dogs. The procedure adopted was briefly as follows: Minute portions of the tumour were implanted through skin incisions into pockets underneath the *panniculus carnosus* of the new host, the operations of excision and implantation being carried out with the most rigid aseptic precautions. Serial inoculations were carried out with both "natural" tumours. From Case 1 the tumour was carried to the eighth generation, when, owing to a vacation occurring, it was allowed to die out. From Case 2 the tumour was successfully propagated to the ninth generation, and is still growing. The structure of the tumour remained constant throughout all generations. The tumours which formed subsequent to inoculation after reaching a period of maximum growth underwent gradual resorption and disappeared spontaneously, or, with one exception, appeared to be doing so, when the animal was killed. In this case (Experiment 2, first series, second generation) the growth gradually increased in size, and, when the animal died, secondary foci were found in various internal organs. In every dog inoculated for the first time tumours developed, except in the case of two puppies inoculated with portions of a nodule the size of a pea, which was all that remained of a large and rapidly disappearing mass of new growth. The age of the animal made no difference to the facility of successful inoculation. Successful results were obtained with young growing tumours, those which had reached maturity, and one which had undergone almost complete resorption. There was no attenuation of the virulence from generation to generation. The larger the piece introduced the larger was the tumour which developed. The effect of the growth upon the animal's general health was in most cases slight,—after an initial loss, the animal regaining its weight again, a permanent slight loss

remaining. The life cycle of the tumours formed after inoculation can best be illustrated by the following examples:—

Serial Inoculation.

Experiment 1.—A young wire-haired fox-terrier bitch (weight, 4629 grms.). Six minute portions of the tumour were implanted into the subcutaneous tissue of the abdomen and flank. The wounds all healed by first intention. On the day following the inoculation the portions of tumour introduced were easily palpable underneath the skin. On the third day the nodules were still palpable, but felt smaller. At the end of a week they could only be felt with difficulty, and seemed to be disappearing. At the end of a fortnight the nodules could no longer be felt, so that it appeared as if the result was to be negative. During the third week, however, minute nodules could be felt at the site of inoculation. From now onwards these gradually increased in size, so that at the end of thirty-eight days four confluent masses could be felt, each of which was the size approximately of a hen's egg. These were painless to the touch, firm in consistence, and the skin was freely movable over them. The tumours reached their maximum limit of growth two months from the date of inoculation. From this date they commenced gradually to disappear, at first slowly, later more rapidly, until, on the eighty-first day they had all entirely disappeared, except one which was reduced to the size of a split pea. This nodule, which was adherent to the skin and had an ulcerating surface, was excised. The weight of the animal remained remarkably constant throughout. There was a very slight loss of weight for the first five days; this was, however, soon made up, and when the tumours had disappeared the animal was found to have lost only 128 grms. in weight.

Experiment 2.—A smooth-haired fox-terrier dog (weight, 5210 grms.). It was inoculated in a similar manner with six pieces of tissue. In this animal the disease ran a somewhat different course. The nodules disappeared to reappear on the tenth day, and on the thirty-eighth day had reached the size of bantams' eggs. On the forty-third the animal, which had been steadily loosing weight, became seriously ill, and as it was at the point of death on the forty-seventh day it was killed. At the post-mortem examination the tumours were found to form six separate masses the size of hens' eggs. They could be shelled out readily from the surrounding tissue. On section they had a uniform hepatic-like consistence, and showed no evidence of necrosis. The regional lymphatic glands were only slightly enlarged. The liver was enlarged, and had scattered over its surface several dark mottled areas. The suprarenals were enlarged, especially the left, which on section showed at its upper pole an opaque grey area. In the middle of the small intestine a small "plaque" was found. This was the size of a florin firmly adherent to the coats of the bowel, firm in consistence, and of a light grey colour.

On microscopical examination the tumours showed (Plate XLIV. Fig. 6) a histological structure which was in the main identical with that found in the natural tumour. Sections from the nodules in the liver showed the portal tracts occupied by young connective-tissue cells, identical with tumour cells, and many were undergoing mitosis. Young capillaries were interspersed throughout, and here and there a leucocyte was seen.

The portal vein was thrombosed, and the adjacent liver cells were destroyed. The nodules in the intestine and suprarenal (Plate XLIV. Figs. 7 and 8) exhibited a structure similar to that of the primary tumours.

The examination showed that the structure of the tumours formed at the site of inoculation (Plate XLIV. Fig 6) was similar to that of the portions of the natural tumours introduced. The appearances in the portal tracts had many points of resemblances to an inflammatory reaction, such as is found in acute interstitial hepatitis.

The localisation of the disease, the great preponderance of the connective-tissue cell, and the very active cell division in these, unaccompanied by the presence of mature fibroblasts, was more in favour of new growth. In this case the animal died with all the characteristics of a malignant neoplasm.

The appearance of the tumours excised at the acme of their growth is shown in (Plate XLIV. Fig. 9) tumours of the fifth generation, second series, seventy-one days after inoculation.

2. *Inoculation into Foxes.*

Two fox-cubs were inoculated with portions of the tumour from the fourth generation of the first series. In both cases tumours gradually developed, increased until they finally reached the size of pigeons' eggs, when the animals died considerably emaciated. At the time when this result was obtained by me no published record of a similar success existed. Two months later, however, Sticker published his second paper, in which he described the development of tumours after inoculation into two foxes.

III. Inoculation into Animals naturally Immune.

Rabbits, rats, and guinea-pigs were inoculated with portions of the tumour without success. In all cases the portion introduced underwent gradual absorption and disappeared.

IV. Inoculation into Dogs with acquired Immunity.

Six dogs in which spontaneous recovery had occurred were reinoculated. In each case the portion introduced disappeared in less than two weeks, and no subsequent tumour formed. This immunity was found to be present from one to eleven months after spontaneous recovery. Five of the animals were inoculated with portions of the tumour from the same strain as that which they themselves had possessed. One animal was reinoculated with both strains, and it was found that immunity acquired to one strain was also present to the other ten months after spontaneous recovery.

V. The Causal Virus.

Cultivations were made from all of the tumours of the first series to endeavour to find the parasite, if such should exist. All the common and many of the more specialised media used in bacteriological work were tried without success. Neither an anaerobic nor aerobic micro-organism could be isolated, except an occasional germ, which was obviously a contamination.

Dr. Ford Robertson kindly examined for me portions of the tumour by his special staining methods, without being able to detect inside the cells the presence of those bodies which he, along with myself, figured

as occurring in human carcinomata, and which possess a certain morphological resemblance to *Plasmodiophora brassica*.

Portions of the tumour were mixed with sterile normal saline solution, pounded in a mortar with sterilised sand, and then filtered through a Berkefeld filter. The fluid obtained was introduced into the subcutaneous tissue of a number of dogs. In none of them did tumours develop either at the site of inoculation or in any part of the body. One of the animals (Experiment 4) died on the seventh week from acute interstitial nephritis.

Films made from the tumours were also examined for the presence of spirochætæ as possible etiological factors, but none were found.

Sticker (1906) failed to find any parasite extra or intracellular. He also found that no tumours developed after the introduction of the filtrate.

San Felice (1904) is the only observer who has found a parasite which he claims to be the pathogenic organism. This is a blastomyces.

Plimmer (1899) failed to find the cell inclusions which he described in human carcinoma.

Beebe and Ewing (1906) found a large spiral organism like the *Spirochæta refringens* in one ulcerating tumour. They do not suggest that it was at all likely to have been the cause of the tumour formation.

VI. The Life Cycle of the Tumours produced by Inoculation.

At the very outset of my investigations into the nature of this disease it became apparent that, in order to determine the exact relationship which it bore to cancer, as we know it, it would be necessary to study the life-history of the tumour from its inception to its final disappearance. At that time no record existed of the process by which the tumour originated, grew, and finally disappeared. Since then the results of a certain amount of work on this subject have been published.

1. *Histogenesis.*

Bashford (1905) is of the opinion that "the establishment of a new tumour is accompanied by a transformation of the connective-tissue cells at the site of inoculation into elements indistinguishable from those of the primary growth. The tumour cells introduced degenerate very rapidly, a few only retaining their vitality for a period of not more than three or four days." He considers it entirely an infective process, "which is identical with that which occurs after inoculation with isolated pathogenic organisms."

Sticker (1904). According to this observer the tissues of the host at no time and in no place take part in the formation of the tumour, which arises by a process of grafting.

Beebe and Ewing (1906) hold that the tumours develop from the cells introduced. That some tissue cells may be included in the tumour or transformed into tumour cells they are not prepared to deny, although there was no evidence of such a process in their specimens.

That these diametrically opposed views should exist is not difficult to understand, when one remembers that we are here dealing with a structure the cell of which is identical with those found in the process of repair round any foreign body.

In order to determine the exact mode of origin of the tumours the scheme of investigation was carried through as follows:—

The tissues of dogs, killed when in apparent health, were examined. Portions of a growing infective sarcoma were excised, inoculated into other dogs, and removed after being six and three-quarter hours, eighteen hours, twenty-four hours, three days, eight days, ten days, fifteen days, and twenty-one days in the tissues of the new host. Portions were also removed when the growth was at its summit, during its declension, and just prior to its final disappearance. The reaction which occurred round inert foreign bodies was examined as a control. Portions of a true sarcoma excised from the leg of a greyhound were implanted, and later excised and examined. The changes which occur in dogs with acquired immunity were also studied, and the cytological explanation of immunity compared with those associated with the histogenesis of the tumour. When the changes which occur in the earlier hours were being studied it was found necessary to destroy the animal. The skin, subcutaneous tissue, and deep fascia were then removed in one layer, fixed for a few hours in saturated corrosive sublimate solution, and then sectioned to allow the fixative to penetrate (Plate XLIV. Fig. 10). By such means an excellent opportunity of examining the relationships of the growth could be obtained.

Certain fallacies had to be avoided. In the carrying through of the specimen, owing to the shrinkage of the tissues which occurred, retraction of the nodule and the tissues of the host took place at some parts. This was liable to leave fragments of the tissue of the nodule adherent to the tissue of the host, and, on the other hand, loose tags of areolar tissue were apt to remain fixed to the outer side of the nodule introduced, and might be mistaken for portions of the capsule of the tumour introduced at the time of inoculation. In order to make this point clear, in every case where such a fallacy was liable to occur, the nodule was sectioned completely, and serial sections of its entire bulk examined. This point, as will be seen later, is of special importance, for in the majority of cases, in the area from which the tumour grows, a process of immediate union between the tissues of the host and those of the nodule is present, with no fibrinous zone to demark a boundary. Control inoculations were, of course, made in every case into other dogs. Tumours developed in every one of these.

From six hours up to fifteen days certain well-marked changes could always be seen at the site of implantation.

1. The centre of the nodule showed in every case evidence of cell degeneration, and ultimately necrosis.

2. Farther out, close to the periphery of the nodule, there was present a zone where leucocytes—at first mainly of the polymorphonuclear variety—abounded.

3. Beyond that, but still in the substance of the nodule, there was a zone where, after twenty-four hours, large mononuclear phagocytes could be seen removing the necrotic tumour tissue.

4. Beyond this there was present in every case a zone which was directly continuous with the boundary zone, and usually continued into the tissue of the host, where healthy tumour cells could be detected—"the tumour formation area."

5. External to this the tissue of the host showed active reaction.

6. In addition, at some parts the tissues of the host and the nodule introduced could be seen to be separated by a layer containing fibrin and cells of the inflammatory exudate.

The examination of the nodules removed, after having been in the tissues of the new host for from six hours to fifteen days, shows clearly how difficult it is to determine the exact process by which the new tumour arises. This is found to be due to the fact that "the tumour formation area" is situated in every case where the tumour cells of the portion introduced are directly in contact with the tissue of the host, where in fact a zone of immediate union exists. When a portion of fibrous tissue forms the periphery of the fragment inoculated, and a process of immediate union has occurred, no such zone of tumour formation develops. Where haemorrhage or abundant exudate separates the tumour cells of the nodule from the tissue of the host, in a similar manner no definite zone of tumour growth exists. As early as eighteen hours after inoculation the tumour formation area is definitely created (Plate XLII. Figs. 1 and 2). On one side it gradually merges into what is obviously necrotic tissue belonging to the portion introduced, and on the other side it extends into the tissue of the host with no boundary zone to demark the line of junction.

The reaction which the presence of the nodule of new growth excites in the tissue of the new host follows the lines, as one would naturally expect, of the cardinal changes associated with the process of repair or inflammation.

Changes in the tissues of the host.—At six hours the vessels of the surrounding tissue are seen to be much engorged, and through their walls leucocytes are seen to be emigrating. These cells are also abundant in the extravascular tissues, and correspond closely in their variety and proportion to the result obtained by Beattie (1902) in his study of the inflammatory exudate present after the intraperitoneal injection of *Bacillus coli communis*. Polymorphonuclear leucocytes are most abundant, mononucleated cells — lymphocytes and mononucleated leucocytes—are also seen, in addition to an occasional eosinophile leucocyte. The situation in which these cells are found is of interest. In the adjacent tissue of the host and in the layer of fibrinous exudate the customary relative proportion is maintained. Deep in the area spoken of as the zone of leucocyte invasion the polymorphonuclear leucocyte alone is present. The mononucleated cells are also seen to have emigrated into the nodule by six hours; but they have not penetrated so deeply, being confined to the periphery of the necrotic tissue. This state of affairs is very similar to that obtained by Maximow, who found, in the centre of the capsules he introduced into animals, that the polymorphonuclear leucocyte was alone present at first. The presence of the polymorphonuclear leucocyte is associated with a concomitant cell degeneration in the zone of leucocyte invasion of the necrotic tumour tissue.

The cells of the inflammatory exudate are virtually absent from the tumour formation area, and this would appear to be due to a

negative chemiotactic influence on the part of the living tumour cell, as through this area they have to pass to reach the necrotic tissue beneath, in which they are abundant.

In the later experiments the relative proportion of the various cells seen in the necrotic tissue at various periods corresponds very closely with that proven by Beattie to be the case in non-fatal cases of peritonitis. The polymorphonuclear leucocyte is most abundant up to three days. It then gradually diminishes, and is virtually entirely absent by the eighth day. The large mononucleated cell of Ehrlich is especially seen from twenty-four hours to the third day. The mononucleated cell of the lymphocyte type, and its further derivative the polyblast are seen at six hours, are abundant at twenty-four hours, and are the most important cells present during the histogenesis, growth, and disappearance of the tumour. The source of the polyblast would appear at first to be undoubtedly almost entirely from lymphocytes emigrated from the blood stream, as Marschalko, Maximow, and others maintain. At a later period, during the growth of the tumour and its disappearance, there will be seen to be evidence of the arrival of these cells by the lymphatics.

Reaction on the part of the fixed tissue cells.—Neither the operation of introduction nor the presence of the foreign body caused death of the surrounding cells to any appreciable extent. At six hours the adjacent connective-tissue cells are swollen up, and have an appearance similar to that seen prior to cell division. The endothelial cells show a similar change. It is probable that already young fibroblasts have been formed by direct (and in one case observed, by indirect) division; but this is not capable of absolute proof. The changes which occur round inert foreign bodies correspond closely to those described. If the formation of fibroblasts has occurred at six hours, this is earlier than is found in such cases. Kiener and Duclert (1893), however, describe a similar early fibroblast formation.

At eighteen hours the formation of fibroblasts and transition forms from these to tumour cells can be seen, and young endothelial cells are already present in the outer part of the tumour formation area (Plate XLII. Fig. 2). At three days the formation of young fibroblasts and the development of tumour cells from them is proceeding most actively in the tissues of the host, mitotic figures in many of them being visible. Young capillaries can also be seen at this period growing from the tissue of the host into the tumour-formation area.

At eight days the adjacent areolar tissue of the host is found to be invaded by tumour cells, the connective-tissue cells are increased in number, and show transition forms to tumour cells. Mitosis in the former has been seen, but is not now commonly found (Plate XLIV. Fig. 11).

Changes in the nodule introduced.—In every case the centre of the nodule showed early and extensive degeneration. This affects all the component parts of the tissue introduced, but is most evident in the tumour cells. At six and three-quarter hours the tumour cells at the periphery of the nodule appear healthy where they are in contact with the tissue of the host. The crucial period in the cytological evolution of the new tumour is undoubtedly the first few hours after inoculation. At eighteen hours the peripheral zone of healthy tumour cells is very distinct where immediate union has occurred (Plate XLII. Figs. 1, 2). Healthy tumour cells which are undoubtedly derived from the tissue introduced are visible, and many of them are undergoing mitosis. Where a thick layer of fibrin is present an occasional tumour cell of the portion introduced is seen undergoing division; but these are very scanty in number as compared with the area of intimate union. Already the cells from two sources have merged. At no period can complete death of the tumour cells of the nodule introduced be made out associated with the creation of a tumour formation area definitely within the tissues of the host. At three days the tumour cells in the tumour formation area are very abundant, and many of them are undergoing mitosis. At eight days they are very abundant, and tripolar mitosis has been seen. The area has now acquired the structure characteristic of the natural tumour, and is invading the adjacent areolar tissue. At fifteen days it is flooding the tissues of the host, extending along the perivascular lymphatics, and cutting off islands of dense fibrous tissue, which by this means become included inside the developing tumour (Plate XLV. Fig. 12).

Conclusions regarding the histogenesis of the tumour.—The tumour formation area is present only where tumour cells of the nodule introduced are directly in contact with the tissues of the host from the first. Here tumours form. At other parts granulation tissue develops between the two surfaces. At eighteen hours, twenty-four hours, and three days there are seen cells which are identical in appearance, but are obviously derived from two sources—tumour cells of the nodule introduced, and similar cells from the tissue of the host. Active mitosis is noticed to be occurring in both varieties.

The tumour is thus seen to be formed by certain of the peripheral cells of the tissue introduced, living and actively dividing, and at the same time altering the process of repair in the surrounding connective-tissue cells; so that instead of going on to form mature fibroblasts, they remain immature and continue to divide actively, having acquired the characters of a sarcoma cell, as which they live and ultimately die without ever again developing into mature fibroblasts or forming fibrous tissue. The virus is one which is conveyed only by direct contagion. How far each source of the tumour cells is responsible for the tumour ultimately formed is impossible to determine. The capillary stroma of the tumour is created from the tissue of the host.

I thus cannot agree entirely with Bashford, whose opinion I at one time was inclined to homologate. I fail to see how he is able to state that the tumour cells introduced degenerate very rapidly, a few only retaining their vitality for a period of not more than three or four days. I also cannot agree with Sticker when he claims for the tumour an origin entirely from the nodule introduced, and states that the tissues of the host in no way take part in the creation of new tumours. I agree with Beebe and Ewing when they state that the tumour is formed from the cells of the nodule introduced; but instead of only considering it possible that the growth may be added to by new cells from the tissues of the host, I would claim that the stroma is formed from this source, and that, to a very considerable extent, this also serves to act as a source of new tumour cells.

2. *Growth, Resorption, Disappearance.*

The only published records which exist of the *later stages of the life cycle of the tumour* are—

1. The brief note by Smith and Washbourn (1898), who say that "the tumours formed disappear by ulceration."

2. The opinion expressed by Sticker (1906), who, after describing the hæmorrhages frequently met with in the disappearing tumours, attributes to them the disappearance of the growth.

The process by which growth, resorption, and disappearance of the tumour took place was investigated by examining nodules removed from dogs when the tumour was increasing in size, when it had reached its apparent maximum, during its declension, and just prior to its final disappearance. In addition to comparing the changes which occurred in different animals, the process was observed in one case from time to time from the third day to just prior to the final disappearance of the growth on the one hundredth and eighteenth day.

Growth.—At fifteen days the tumours were definitely established. Nodules removed on the twenty-second day were found to have increased much in size. They appeared to be encapsulated, and had the uniform fleshy consistence characteristic of the tumour. In none of them could evidence of the necrotic tissue of the portion introduced be seen. On microscopical examination the most noticeable feature was the number of tumour cells undergoing division; as many as ten such cells were visible in a single field. Tripolar mitosis was not uncommon. In contact with the capillary stroma throughout the tumour an occasional lymphocyte was seen (Plate XLV. Fig. 13).

Very occasionally an eosinophile leucocyte was seen amidst the tumour cells. The periphery of the tumour was penetrated by young blood vessels which contained an increased number of

lymphocytes. At the outer border of the tumour the flooding of the surrounding tissue by the tumour cells was no longer apparent. An increased number of fibroblasts, some of which were in mitosis, were here seen (Plate XLV. Fig. 14). These, however, instead of showing transition forms to tumour cells, were mostly drawn out, spindle-shaped cells, and were arranged with their long axis parallel to the outer border of the tumour. In this region the lymphocytes and polyblasts were more abundant.

Resorption.—A nodule examined at seventy-four days when tumours which had reached the size of billiard balls had been stationary for a fortnight was found to possess the structure of the natural tumour. Mitosis was present, but not so abundant. The periphery of the tumour was made up of a laminated border of fibrous tissue. In this lymphocytes, polyblasts, and an occasional degenerating tumour cell were seen, and a few polyblasts were visible settling down as sessile adventitia cells. Throughout the tumour lymphocytes, polyblasts, and eosinophile leucocytes were present in increased number, the second variety being especially evident where degenerating tumour cells were situated. Several hæmorrhages were seen.

Disappearance.—The examination of a nodule removed from an animal in which several had already just disappeared showed (Plate XLV. Fig. 15) a minute nodule of tissue surrounded by a thick laminated border of dense fibrous tissue in which many polyblasts and degenerating tumour cells were apparent (Plate XLV. Fig. 16). The centre of the nodule appeared bright red in sections stained with P. R. Mg. owing to the number of polyblasts (Plate XLIII. Fig. 3). An occasional tumour cell can be found. None are undergoing cell division; all are degenerating.

On examination of the site whence a nodule had been absorbed the tissue was found to consist of wavy strands of fibrous tissue, between which at certain parts a few lymphocytes and their derivatives could be seen.

The growth of the tumour from the twenty-second day onward is accounted for by division of the tumour cells already formed. The vast majority of the young fibroblasts now formed proceed to the formation of fibrous tissue, by which means the encapsulation of the tumour is brought about.

At seventy-four days resorption has commenced. The tumour is definitely encapsulated. The formation of additional tumour cells continues to take place from the surrounding tissue until the reaction of the tissues of the host is such that it is able to resist the action of the virus.

The final disappearance of the tumour is associated with a flooding of the tumour tissue with lymphocytes, polyblasts, and plasma cells. The tumour is borne away on a lymphocyte tide which, as it recedes, may leave a few of its number as sessile adventitia cells.

Examination of nodules disappearing by ulceration shows the ulceration to be a mere coincidence dependent upon a previous involvement of the skin.

VII. The Life-History of the Nodule inoculated into Animals with acquired Immunity.

We have already seen how, in six of the animals in which spontaneous recovery had occurred, no tumours formed on subsequent inoculation. This result is in accordance with those obtained by Smith and Washbourn and Sticker. The former with four such dogs and the latter with twenty failed in every case to produce a tumour. Bashford, however, says: "A small proportion of growths, after having attained a certain size, diminish in volume and ultimately disappear; such recovery has not been followed by an immunity to subsequent inoculation, and animals with large tumours have been successfully reinoculated." In the light of the results obtained by other investigators his statement concerning the absence of immunity in his experience is extremely difficult of interpretation.

In studying the changes which occur after inoculation into animals in which spontaneous recovery had taken place, portions of the tumour were inoculated into the subcutaneous tissue and excised after one, three, seven, and ten days. The only weak link in the chain of evidence was in the three days' experiment, in which case the animal was killed, and consequently the disappearance of the control nodules could not be observed as in all the other cases.

The process by which immunity is established is, from the appearances observed, seen to be one which may be aptly compared to the complete life cycle of the tumour which develops in an animal which is not immunised; instead of occupying four months, it is in this case finished in less than a fortnight.

The nodule introduced lives. The tumour cells may divide. It is almost at once flooded with cells of the lymphocyte type and polyblasts which, when abundant, are always associated with the presence of degenerating and disintegrating tumour cells, and the surrounding tissue of the host proceeds at once to the formation of mature fibroblasts, which invade and encapsulate the nodule. This is, so far as I can ascertain, the only record that exists of the cytological changes which occur in such animals.

A comparison of this process was obtained with that which occurs when a true malignant neoplasm is inoculated, and, as is the rule, fails to take. A rapidly growing fibro-sarcoma from the limb of a greyhound was inoculated into a fox-terrier. All of the portions inoculated failed to develop tumours. On examination a central zone of necrosis was seen, and a zone of peripheral invasion by cells mainly of the lymphocyte type, and outside this a zone of laminated

granulation tissue. In this, fibroblasts, forming fibrous tissue with some lymphocytes and polyblasts, were situated. The latter were, however, not so abundant as in the immune dog inoculated with infective sarcoma.

VIII. Changes in the Blood during the Growth of the Tumour *In the Dog.*

It was obvious, when the lymphocyte and its derivatives were found to play so important a part in the life cycle of the tumour, that a careful examination of the blood at stated intervals would give facts worthy of note. This was done.

The blood of eight animals was examined. As an illustrative case the following will suffice:—

Experiment 29 (Sixth generation, second series).—*A large brown bull terrier.*—The blood of this animal was examined on the day prior to inoculation, and at regular intervals for one month. At the end of this time the tumours had grown to masses as large as pigeon's eggs. The accompanying chart shows how an initial leucocytosis occurred which rapidly subsided. After this the total leucocyte count remained fairly constant at the normal figure. The percentage count, however, showed marked alteration. A marked diminution of the polymorphonuclear leucocytes with a corresponding increase of the mononucleated cells, and also of the eosinophile cells, took place.

Experiment.—*Fox cub*, No 2.—This animal had at the time of the first examination small tumours developed. The blood examination was conducted for forty-seven days. The total leucocyte count remained virtually uniformly constant at 10,000 per c.mm. The percentage count showed marked alteration. At the first examination there were present 58 per cent. mononucleated cells, 40 per cent. polymorphonuclear leucocytes, and 2 per cent. eosinophiles. From this date a gradual increase took place until the eleventh day. A gradual alteration now took place, the relative proportion of polymorphonuclear cells increasing and the mononucleated cells diminishing until the time of death.

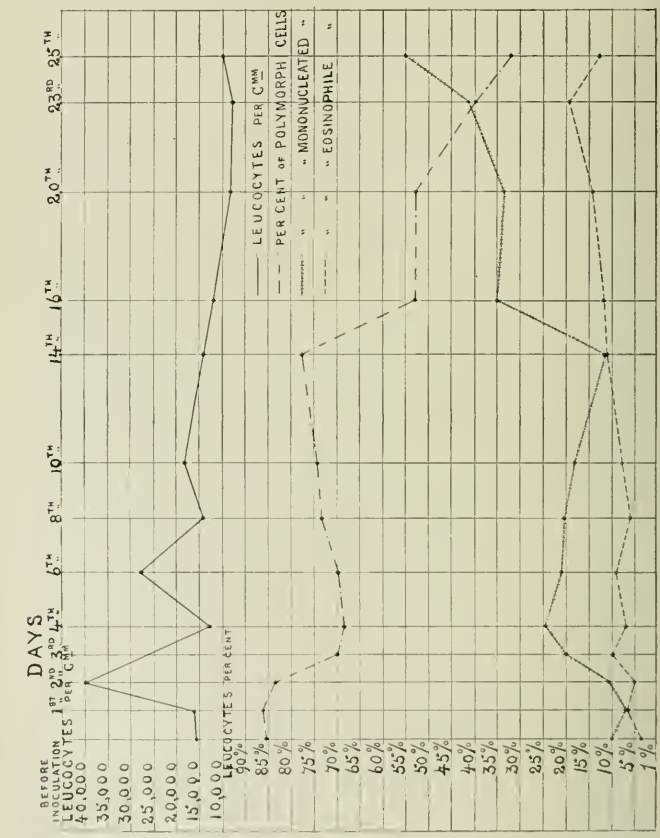
Natural tumour, Case 2.—The tumours present on the penis had been excised three months previously and were almost invisible. Its blood was examined on four occasions during two months, and practically no variation occurred. The average of lymphocytes and mononucleated cells was found to be slightly higher than is the case in the normal dog.

The establishment and growth of the tumour is accompanied by a steady increase in the percentage of mononucleated cells in the circulating blood.

In the other animals examined evidence of this progressive lymphocytosis associated with the growth of the tumour was also seen.

I am indebted for the above facts to Dr. J. M. Graham, who kindly conducted, at my request, careful daily examinations of the blood of the animals. The investigation extended over a period of two months. The record of his work is of twofold value, as, not only is it that of an accurate and skilful observer, but also of

one who approached the subject with a perfectly unbiased mind, as he was unaware that a lymphocytosis was to be expected in the cases under investigation.



EXPERIMENT 29.—Record of Blood Examination.

IX. Discussion of the General Life-History of the Tumours formed after Inoculation, and a Comparison of it with the Process of Repair.

The histogenesis of the tumour has already been shown to be caused by a grafting of tumour cells, of the portion introduced, upon the tissues of the new host, associated with a concomitant formation of

similar cells from the latter source. This change has been seen to occur only in those situations where direct contact is present. The tumour cells created in the tissue of the host are formed by anaplasia from the fibroblasts present in the natural process of repair round any foreign body. When we compare the changes in the fixed tissue cells adjacent to the nodule introduced with that seen in the response to certain other irritants, we noted in both cases a rapid response on the part of the connective tissue and endothelial cells, which varies in rapidity of appearance and degree according to the intensity of the virus. In the reaction of the fixed tissue cells to a definite infective process, such as a micro-organismal infection, there can be seen, after twenty-four hours, according to Maximow, a swelling of the protoplasm of the connective tissue cells, especially those in the neighbourhood of the blood vessels, an alteration in the contour of these, whereby the thin flat processes become thick and sharp and their nuclei enlarge and occasionally exhibit mitotic figures. In the course of the first few days fibroblasts are formed in great numbers, which, according to his experience, is not the case in an aseptic inflammation until three to four days had elapsed.

Anaplasia of these fibroblasts is of frequent occurrence after acute infection, and has been noticed to be especially well seen in the dog during the stage of four to five days, and also what would appear to be the undoubted formation of fibroblasts from active proliferation of endothelial cells. This has also been seen by me during the histogenesis of the tumour and in animals which were immune, and would go to support the work of Baumgarten that blood vessel endothelium can produce connective tissue.

The differences which are noticed in the changes associated with the histogenesis of the tumour are—

(1) The formation of the fibroblast would appear to take place earlier, although, as we have already mentioned, Kiener and Duclert (1893) describe their formation after a micro-organismal infection as early as six hours.

(2) The anaplasia occurs earlier, and instead of being a transient reaction it persists, and by the active division of the cells the formation of a mass is brought about, having the histological structure of a tumour, from the centre of which, during its creation and growth, the blood borne cells of the inflammatory exudate can be considered as being virtually absent. The transient anaplasia of the fibroblasts associated with an intense micro-organismal infection passes off, and is followed by organisation, during which there is interspersed throughout these fibroblasts other cells of the inflammatory exudate, especially the mononucleated cells called polyblasts, which we consider to be virtually entirely derived from altered lymphocytes. The rôle of the lymphocyte, and of its further development the polyblast, in the process of repair and histogenesis of the tumour, would appear to be in many respects

similar. In the acute inflammatory process similar mononuclear cells are present in the peritoneal inflammatory exudate, according to Beattie (1902) in increased amount in eighteen to thirty hours. These cells are generally recognised to act as phagocytes to cellular débris, and to a lesser extent to living micro-organisms. It is also a well-recognised fact that in certain infections, such as with the tubercle bacillus, they are especially abundant, and subserve almost entirely the function usually discharged by the polymorphonuclear leucocyte in acute infections.

Maximow, in his work on inflammation produced by the introduction of aseptic foreign bodies, found that an early active emigration of lymphocytes from the blood vessels took place; a cytological development of these occurred leading to the formation of similar large mononucleated cells which arranged themselves around the foreign body, in contact with it, and throughout the fibroblasts later formed round it.

Schwarz (1904) obtained a similar result, and noticed how during the course of the first two hours after the injection of the foreign body into the tissues there appeared a very plentiful and undoubted emigration of lymphocytes from the blood, and the development from these of large mononucleated cells.

Ziegler, K. (1904), in his work on the skin and subcutaneous tissue of human beings, saw a similar emigration of lymphocytes from blood and lymph vessels.

The function of these in acute inflammation is, in certain respects, obvious. Where tissue débris has to be removed they can be seen with particles of this débris ingested by their protoplasm. Where the infection is less intense they appear to be largely responsible for limiting its spread, as, for example, in the production of giant cells and the giant cell system in a tuberculous lesion. In the process of repair round an aseptic foreign body they are present at an early period, but gradually diminish in numbers until only those remain which have become incorporated in the fixed tissue cells of the part, as sessile adventitia cells known as clasmatocytes. This is brought about by a gradual transformation of the cells characterised by an alteration in their size and an increased granularity of their cytoplasm due to the presence of a substance the nature of which is unknown, but which according to Ranvier (1900), who first named them, is a secretion of the cell which may play a part in the nutrition of the tissues. These cells, in his opinion, are unicellular glands like the leucocytes, and their secretion is necessary for tissue metabolism.

Justi (1897) has noticed, especially in tuberculous infection, cells similar to those described as polyblasts, and he thinks that they are probably the carriers of a substance for the nutrition of the rapidly growing cells.

In the histogenesis, growth, and gradual disappearance of the tumour the lymphocyte and polyblast would appear to play a definitely important part. They are from the first found in the nodule only in the areas where necrotic cells exist, and would appear undoubtedly to be responsible for the removal of these. In these regions at a later stage they are associated with mature fibroblasts and definite granulation tissue. With the increased growth of the tumour and its invasion of this region they disappear in this position, and definite fibrous tissue formation never occurs.

From the earliest period it was always a noteworthy fact that in

those areas where healthy tumour cells were found the mononucleated cell was always virtually absent. In the tissues of the host adjacent to the nodule they were present from the first, and at the extending border of the growth they were always to be found.

The examination of the blood would appear to establish the fact that the disease is one which is characterised by an increase of the total number of mononucleated cells, especially of the lymphocyte type in the circulation. Similar cells are also seen, forming extravascular accumulations to blood vessels in the tissues beyond the border of the extending growth. As the tumour gradually forms, these cells become evident in increased numbers inside the vessels entering it, and outside them throughout its substance, where they form lines of extravascular undifferentiated lymphocyte-like cells. At this period they are especially evident at the outer border of the growth, where the fibroblast is now seen to be proceeding to form fibrous tissue, and taking part in the creation of a capsule to the tumour.

The gradual absorption of the growth is one which is most evident at the outer margin where mononucleated cells abound, and their presence here is associated with a gradual degeneration of the tumour cells adjacent to them and the formation of new fibrous-tissue strands. Throughout the tumour during its resorption the mononucleated cells become gradually more evident, and more of them are of the polyblast type. The disappearance of the growth is accompanied by a great increase in the number of these throughout it, and at this period the number present in the adjacent tissue may be so great as to form distinct lymphoid masses. Those, however, which are directly in relationship to the degenerating tumour cells are now almost all of the larger mononucleated type, and very many of them have their protoplasm rich in the material which stains so intensely "basophile," and which is the noticeable feature of the so-called plasma cells. This material stains in a manner similar to that found in the elasmatocyte, and was looked upon there as an intracellular secretion. Phagocytosis of the tumour cells by polyblasts can very occasionally be made out, but this is very exceptional; the process would undoubtedly appear to be one where the tumour cell is degenerated by cytolysis prior to its removal by the phagocytes.

There is a certain amount of evidence in favour of the contention that the destruction of the tumour cells is due to a process of extracellular digestion by the polyblasts. This never occurs in the absence of the latter cells. Where they abound such of the tumour cells as are not obviously degenerating are never seen undergoing mitosis.

Finally, successful inoculation has followed the introduction of a tiny nodule the size of a lentil, which was all that remained of a tumour, but this, however, contained a minute portion of tumour

tissue uninvaded by polyblasts; whereas, on the other hand, failure took place in another case where at a slightly later date the nodule was completely invaded and the tumour cells destroyed. These experiments would appear to support the contention that the cause of cell destruction lies in the invading polyblast and not in the serum of the circulating blood bathing the tumour. The other explanation possible of such a gradually advancing process of cell death is, that the serum contains the cytolysin, which is so scanty in amount that it is used up by the peripheral tumour cells, and the polyblast can only advance against those which are devitalised. The death of the cell cannot be attributed to the loss of its own inherent vitality in view of subsequent immunity produced and the facility of inoculation which they show in the non-immunised dog.

The part which the mononucleated cell plays in the prevention of tumour growth in the immune animal has already been shown. The tumour grafts as before, but is at once invaded by lymphocytes which show a cytological development into polyblasts. The growth is arrested. The cells of it are destroyed and disappear.

X. The Etiology of the Tumour.

The process by which tumours arise in the natural course of the disease has not been worked out in detail. It is, of course, as we have already seen, well known that the infection is conveyed during the act of coition from another animal, with tumours upon its genital organs This is followed after some weeks by the appearance of minute vascular nodules, which develop into tumours that persist much longer, and much less seldom disappear than is the case in those produced by artificial inoculation.

The causal virus is, as already said, unknown.

Those who claim that the tumours formed after inoculation are created entirely from the portion introduced are logically compelled to acknowledge that a similar process must occur with natural infection, which would mean that not only was the sporadic tumour made up of cells which belonged to tissues of the previous generation, but that all the tumours at present in existence were made up of cells derived from some animal which had in the remote ages acquired this disease in a manner which is incapable of explanation, but one which had conferred on the cells formed a power of unlimited growth and proliferation.

Although I am not prepared to deny that such is possible, and that the power of apparently unlimited propagation which the cancer cells of Jensen's tumour possess is evidence in its favour, I would maintain that if this be the natural mode of infection it stands alone as a unique mode of the propagation of disease, which is without analogy in the domains of pathology. When we consider the other

possible explanations of the mode of origin of the tumours we at once realise that the virus is one which is so intimately bound up with the life of the cell that it cannot be dissociated from it by any means known at present, and consequently it would appear that only by the passage of a virus from a living cell to a living cell can the infection be explained. This process must be much akin to that which takes place in the propagation of the disease by artificial inoculation, with this difference, that in the latter case the favourable surroundings allow many of the cells introduced to live and divide, and add to the tumour mass which they have originated and help to form parts. This would explain how in such a case the tumours form much more frequently, grow much more rapidly, become so much larger, and also possibly the much higher percentage of spontaneous recoveries.

If we accept the hypothesis that the processes of natural and artificial infection correspond, with this difference, that in the latter case the process is complicated by certain of the cells of the previous generation living, we may obtain a fair insight into the nature and mode of infection by studying the inception and growth of the tumour. If such will be accepted I would submit that the deductions which follow concerning the etiology of the disease from the facts which we have already established are—

1. The tumour is one which is formed by the reaction of the tissues to a virus which especially affects the fixed tissue cells, leads to the production of fibroblasts in excess, and these by anaplasia become converted into tumour cells, which continue to divide actively and to exert a negatively chemiotactic influence on the mononucleated cells at the site of tumour formation.

2. The response on the part of the body of the host consists in the production of lymphoid cells in excess. The arrest of the growth is due to the creation by the host of a substance which is probably borne in the mononucleated cell, and which at first arrests the further formation of new tumour cells from the fibroblasts of adjacent tissue, and allows these to proceed to carry out their natural function of fibrous tissue formation, and later is responsible for the gradual absorption and disappearance of the tumour by a destruction of its cells with a concomitant formation of fibrous tissue. The mere absence of the mononucleated cell from the zone of tumour formation might explain the continued cell division and the formation of the tumour in a manner similar to that advanced by Ribbert in explanation of the creation of true neoplasms, the formation of which he explains by an isolation of certain of the cells of the body and a loss of restraint normally exerted upon them by their fellows. The fibroblast in one case may, owing to the absence of the mononucleated cell, have been removed from a restraining influence which is naturally present, regulating its development and limiting its growth.

The causal factor may, on the other hand, be an intracellular virus,

which stimulates the cell to active division and repels the mononucleated cell, which only returns when it is the bearer or the follower of a substance which is capable of inhibiting the growth and destroying the life of the tumour cell.

XI. The Renal Changes induced by Infective Sarcoma. Experimental Acute Interstitial Nephritis.

When examining the lesions present in the organs of the second animal which was inoculated, and which died with secondary foci of the disease in certain parts of its body, evidence of acute interstitial nephritis was seen. The important relationship which this inflammatory change bore to the disease was further emphasised when a similar change in a more acute form was detected at the next post-mortem, which was held on a dog that died in the sixth week after the introduction of a filtrate obtained from the tumour. So far none of those who have investigated this disease have described any changes produced by it in the kidneys. To determine how far those found by me could be attributed to the presence of the tumour and the introduction of a filtrate obtained from it, investigation was conducted as follows: The kidneys of six dogs which had been killed outright when in apparent good health were examined. These animals, which were used in the course of an investigation conducted in another place, and which, I was informed, were obtained from the same source of supply as were mine, showed no evidence of recent or former inflammatory change in their kidneys.

Thereafter the kidneys of twenty-three animals were examined. Sixteen of these were dogs which had been inoculated with portions of the tumour. Five were animals into which the filtrate obtained from the tumour had been introduced. One dog which had recovered from the sporadic tumour was examined, and finally the kidneys of one of the foxes which had developed tumours were also investigated. In all of these animals, with three exceptions, evidence of an inflammatory change was present, the apparent age of which corresponded closely with the period during which the virus had been exerting its action. The changes varied in degree, but in all the appearances closely corresponded, and were the manifestations of the same pathological process, with such differences as one would naturally expect to find from the severity of the reaction produced and its duration.

Kidneys from Animals (Experiment No. 2). — Second Generation, First Series.

This animal died forty-seven days after inoculation with portions of the original sporadic tumour, and at the post-mortem examination there was found, in addition to the primary growths, secondary foci of the disease in the liver, intestine, and suprarenal gland.

Morbid Anatomy.—The kidneys showed no obvious change. *The right*

was somewhat bulky. Its capsule stripped readily, exposing a surface which was smooth and of a pale somewhat mottled appearance. On section it was of the usual consistence, and its cut surface also appeared pale in colour. The cortex was somewhat swollen, and the vascular marking was not distinctly visible. Opaque strands could be seen, especially at the boundary zone, extending as faint lines into the cortex.

The renal pelvis showed no evidence of disease, and the larger vascular trunks did not appear thickened. No evidence of amyloid degeneration was present to the naked eye or revealed on testing with iodine solution.

Histological Structure.—The most noticeable feature on microscopical examination was the large number of lymphocyte-like cells throughout the interstitial tissue of the organ. These were especially seen at the boundary zone as large extravascular accumulations, following the lines of the interlobular vessels throughout the cortex. This appearance was not constant for the whole kidney, but was confined to zones which radiated through the cortex. The blood vessels showed no evidence of disease of their walls. The glomeruli showed little alteration,—those which showed the most definite change were situated in the zones where small cells were abundant. The afferent and efferent arterioles showed no evidence of hyaline degeneration of their walls. The glomerular capillaries showed no evidence of disease. Bowman's capsule in some cases was the site of a distinct reaction; the endothelial cells lining it were swollen up, their nuclei being much more distinct, and it was evident that recent proliferation of these had taken place. In the zones where the small cells were most abundant the reaction on the part of Bowman's capsule was most evident. Apart from the proliferation of the endothelial cells, little further change might be noticed. In some cases, however, the capsule was seen to be swollen—to be distinctly fibrillated, minute spindle-shaped cells being arranged around it in a concentric manner. The tubules were, for the most part, healthy. Fatty degeneration could, however, be made out to have occurred in certain of those included in the zones of reaction. The cells in the interstitial tissue were found, on examination, to be mostly polyblasts and plasma cells, and some undifferentiated lymphocytes. Mature spindle-shaped fibroblasts could not be detected, although it would appear probable that certain cells which were present, which appeared like tumour cells, were of connective tissue, and probably also endothelial origin. The left kidney was found, on examination, to manifest changes of a similar nature.

From the foregoing facts it would appear that there was here present a recent acute interstitial nephritis, with an associated slight parenchymatous degeneration mainly affecting the ascending limbs of the looped tubules of Henlé.

Examination of the Kidney of the Dog (Experiment No. I).—Second Generation, First Series.

In this animal the tumours developed and then gradually diminished in size, and finally disappeared eighty-one days after the date of inoculation. Four months later it was again inoculated, but no tumours formed. A year and a half from the first inoculation portions of the stock of a second natural tumour were introduced into it with a similar result, and the animal was killed ten days later, as these were disappearing—that is, approximately a year and a half from the date of the original experiment.

Morbid Anatomy.—At the post-mortem examination the kidneys were seen to be small; in both numerous subcapsular cysts existed. The capsules were thickened, somewhat adherent, and when removed revealed an irregular surface with a morocco-leather appearance. The organs were firm on section, and the cortex was atrophied. The vascular markings were irregular in their distribution, and the surface had a general diffuse, granular appearance through-

out. The pelvis was healthy. The larger vascular trunks were not noticeably thickened.

Histological Structure.—On microscopical examination the capsule is seen to be united to the kidney cortex, under the depressions on its surface, by bands of scar tissue, which pass down to the boundary zone. These have somewhat the appearance and general distribution that was noticed in the previous case. The zones here, however, in contradistinction to what was found in the former, traverse the cortex to its surface more frequently, and are obviously much more fibrous, having included inside some of them degenerated tubules and obliterated fibroid glomeruli.

Blood Vessels.—The larger vessels show some thickening of their outer coats, due to an increase of fibrous tissue.

Glomeruli.—Those situated in the zones of healthy cortex appear normal, whereas of such as are included in the zones of scar tissue some are distinctly fibroid, others show changes present in them to a lesser degree. In the latter the afferent and efferent arterioles show no evidence of disease. In the glomerular tuft there is no change present in the walls of the capillaries forming it, and its nuclei are not increased in number. The capsular space is occupied by a granular albuminous exudate, into which in many cases an occasional red blood cell has escaped.

Bowman's capsule is usually distinctly fibrillated by layers of concentrically arranged cells with delicate spindle-shaped nuclei. The endothelial cells lining the capsule are in some cases swollen and distinct. The periglomerular tissue consists, in many, of concentric layers of delicate fibrous tissue, with small deeply staining cells which have deeply staining nuclei between the fibres.

The tubules show fatty degenaration in the secreting tubules, in relationship to the zones of scar tissue. In the tubules not included in these, however, fatty degeneration is occasionally seen, whereas in some others the swelling of the protoplasm with other appearances indicate the presence of cloudy swelling. In the collecting tubules, in connection with the zones of scar tissue, numerous dark staining granular casts are situated.

Interstitial tissue.—Bands of fibrous tissue pass from the vessels at the bases of the pyramids to the cortex, which is tacked down by them. These follow the lines of the interlobular arteries, and include within them degenerating tubules and an occasional fibroid glomerulus. Some of these are more cellular, whereas others are more fibrous. And in them at some parts extravascular accumulations of small cells, with a more or less spherical contour, exist; these consist of lymphocytes, polyblasts, and plasma cells, with some fibroblasts and endothelial cells between them. From these regions they extend between the adjacent tubules, and around the glomeruli, where the two latter cells are much more scanty. With H. Ia. Pf. the fibrous tissue is especially well demonstrated, as it radiates through the cortex and forms periglomerular rings of fibrous tissue, some of which have proceeded further to distinct fibroid glomeruli. It is also seen surrounding the interlobular arteries with a fibrous periarteritis, an appearance very similar to that seen in the last case; but instead of consisting, as it did there, of an aggregation of individual cellular units, it is now made up of organised tissues.

The result of the examination would here appear to demonstrate the presence of a chronic interstitial nephritis, with the addition of a more recent infection.

Examination of the Kidneys of the Dog (Experiment No. 4; Filtration Experiment No. 1).

This animal was a healthy wire-haired fox-terrier, and into it the filtrate obtained from the tumours of the second generation, which developed in the

dog (Experiment No. 2), was introduced. The process by which this filtrate was obtained was as follows: The tumour was excised, and three nodules the size of small hen's eggs were introduced into a mortar which had previously been sterilised, and sterilised normal saline solution was added. They were then teased out with sterilised glass rods and further broken down with the pestle. The emulsion thus obtained was passed through a new Berkefeld filter. After an hour had elapsed 25 c.c. of straw-coloured fluid was obtained, and this was introduced directly into the subcutaneous tissue of the animal, the whole process being completed in less than two hours from the time at which the tumours were excised.

The amount injected may be, approximately, said to amount to the fluid extract of two-thirds of the tumour bulk used. The effect upon the animal was slight at first. It was kept under especially close observation, as the experiment was conducted to see whether or not the tumours formed might not be due to the presence of an ultra-microscopical parasite, capable, like some others, of passing through the pores of a Berkefeld filter. As has been already stated, no tumours did form. The animal, however, steadily lost weight. Absolutely no local reaction at the site of hypodermic injection could be detected. It continued to lose weight more rapidly towards the end, and finally died six weeks from the date of the original and only injection. A complete post-mortem examination was at once conducted. The appearance detected in the other organs of the body consisted solely of the evidence of a doubtful reaction to a possible toxin, and no cause of death could be made out until the kidneys came to be examined.

Morbid Anatomy.—Both kidneys were of normal size, and had under their capsule minute white points which had somewhat the appearance of miliary tubercles.

The capsule was not adherent, and stripped readily, exposing a surface which was smooth and studded with minute, almost microscopical points, with an appearance somewhat like miliary tubercles.

On section *the cortex* was seen to be of normal diameter. The vascular markings appeared, if anything, somewhat irregular. The glomeruli were not distinct, and no hæmorrhages were present. At certain parts minute grey lines were seen, which followed the direction of the interlobular vessels and obscured them, and could often be made out extending to the minute white specks underneath the capsule.

The Pelvis was healthy, and the larger renal vessels were not apparently diseased.

Histological Structure.—The most noticeable feature was the presence of small cells in the interstitial tissue throughout the organ (Plate XLVI. Fig. 17). These were seen to radiate throughout the substance of the kidney, following the lines of the blood vessels. They commenced around the vessels in the boundary zone and passed to the cortex, where they formed, at some parts, small distinct, wedge-shaped areas, made up of deeply staining, somewhat spherical small cells. The glomeruli showed, in most cases, evidence of reaction. The afferent and efferent arterioles manifested no evidence of hyaline degeneration of their walls, nor blocking of their lumen by cellular increase. The glomerular capillaries showed virtually no reaction. In Bowman's capsule the endothelial lining was usually unduly distinct, its cells being swollen up and increased in number. The capsule itself was often distinctly fibrillated, and could be seen to be made up of small spindle-shaped cells, concentrically arranged; and where this was evident an associated periglomerular accumulation of small cells could be seen. The appearance above described was seen in many glomeruli. In others it was present in a greater or less degree (Plate XLVI. Fig. 18). The secreting tubules showed fatty degeneration, especially in the ascending limbs of the looped tubules of Henlé. This change was most evident in those supplied by the glomerular

unit involved in the developed cicatrix. The cells present in the interstitial tissue were mainly polyblasts and plasma cells, a few lymphocytes and endothelial and connective-tissue cells. An examination of the kidneys in this case showed that they were the site of recent acute interstitial nephritis, with the customary associated slight parenchymatous degeneration.

The complete post-mortem examination revealed no other cause of death. The animal appeared in perfect health when first seen. Since then the only possible toxin which would produce such changes was that introduced. The anæsthetic, for obvious reasons, could in no way have had anything to do with the production of the changes. With these facts before us we are compelled to accept the cause of death as being the toxin obtained by filtration from infective sarcoma tumours, which had induced the acute inflammatory changes, the stress of which had mainly fallen on the interstitial tissue of the kidney and produced acute interstitial nephritis.

The essential nature of the lesions present in the three cases which have been described is the extreme degree of interstitial change associated with a very limited amount of involvement of the parenchymatous tissue; this similarity being especially noticeable in the first and third cases quoted. In these the lesions were seen to be virtually identical. They have been shown to have occurred in animals, both of which succumbed to the effects of a virus to which they had been exposed for a relatively similar period, and in one case to have developed after the introduction of a portion of the tumour; in the other to have followed the introduction of a fluid obtained by filtration for the same.

The lesion of the kidney which they displayed in common is identical with that described by Councilman (1897, 1898) as acute interstitial nephritis. This form of nephritis was, according to him, first described by Wagner under the name of lymphomatous nephritis, and is a variety of kidney inflammation which is frequently the result of acute infective fevers, being found more especially in associationship with diphtheria and scarlet fever. It has been generally looked upon as arising from the operation of a soluble toxin carried to that organ in the blood stream, which leads at the same time to a degeneration of the parenchyma and proliferation of the interstitial tissue, whereof the essential lesions are in the interstitial tissue, and are so prominent and accompanied by so little change in the parenchyma that they cannot be regarded as secondary to parenchymatous lesions. These may be followed by a complete restoration of the integrity of the kidney, or by a gradual organisation of the inflammatory tissue present in the stroma and the development of chronic interstitial nephritis. This would appear to have been the case in the second animal. In the cases we have examined the reaction present in the parenchymatous tissue has been even less severe than is usually met in those described by Councilman as acute interstitial nephritis, with this corresponding advantage, that the changes which occur in the interstitial tissue can be more readily examined.

Summary of the Results of the Examination of the Kidneys.

(*A*) **Dogs inoculated with the Tumour.**

The kidneys of sixteen animals were examined.

One died as described with acute interstitial nephritis.

Five were killed after the tumours had disappeared; four of these were suffering from chronic interstitial nephritis. One showed no evidence of this, and the opportunity of examining for fatty degeneration was unfortunately lost.

Seven were killed when the growth was mainly at its summit; of these—four showed definite interstitial nephritis, subacute in type; two showed fatty degeneration only; and the last one was not examined for fatty change.

Three were examined during the stage of histogenesis. At eighteen hours the kidney showed congestion of the vessels, and slight cloudy swelling of the epithelium of the secreting tubules. At twenty-four hours distinct cloudy swelling was apparent, but no fatty degeneration. Three days after inoculation early acute interstitial nephritis was present. The cortex and medulla were infiltrated at certain parts with numerous lymphocyte-like cells, and fatty degeneration was visible mainly in the ascending limbs of Henlé's tubules (Plate XLVI. Figs. 21 and 22).

(*B*) **Dogs inoculated with the Filtrate.**

The filtrate was injected into five animals.

One died as described from acute interstitial nephritis on the thirty-sixth day.

One was killed after one hundred and nineteen days, and showed advanced chronic interstitial nephritis (Plate XLVI. Figs 19 and 20).

Two were killed after forty-one days and showed fatty degeneration, and in one slight interstitial change.

One was killed after fifty-one days, and showed no obvious change in its kidneys.

The animals which lived showed virtually no reaction in their body weight, except in the case of the one which showed chronic interstitial change. This one lost weight steadily for the first three and a half weeks and then gradually regained it again, so that it was looked upon when killed as a negative result until the kidneys were seen.

(*C*) **Natural Tumour** (Case 2).

This showed slight interstitial nephritis.

The constancy with which these changes occurred justifies the assumption, in my mind, that in many cases, especially after inoculation with portions of the tumour, infective sarcoma causes mild interstitial nephritis.

The General Outline of the Changes produced.

The toxin appears to be one which shows its effects earliest upon the epithelium of the secreting tubules. At first it causes cloudy swelling, and later fatty degeneration of the epithelium, more especially that lining the ascending looped tubules of Henlé. This is definitely evident by three days. By this date also there appears evidence of reaction on the part of the interstitial tissue. This may go on to the production of definite acute interstitial nephritis, or may rapidly pass away. The evidence of necrobiosis in the parenchyma persists, however, considerably longer, but it may also pass off and leave no trace of its presence. A noticeable feature in the morbid anatomy of the kidneys was the frequency with which no positive evidence of inflammatory or degenerative changes could be detected with the naked eye. The changes induced in the glomerular structure were very slight. The glomerular vessels, at no time, could be seen undergoing hyaline degeneration, and never were thrombosed in the earlier cases. A very occasional hæmorrhage could, however, be made out to have occurred from them. The capsular space in many contained an albuminous exudate with an occasional red blood cell in it. In Bowman's capsule the most noticeable feature was the early evident reaction of the endothelial cells lining it. They readily became swollen, and frequently multiplied. Apart from this, the reaction was often slight, but in the capsule itself hyaline swelling could be detected in the earlier cases, and the membrane was noticed in some, swollen up and fibrillated, having a laminated appearance, with distinct nuclei between the fibres. Fatty change would appear to occur in some of these fibrils. The glomerular epithelium showed no constant reaction. In addition to no thrombosis, no obstructive vascular changes could be made out to account in any way for such reaction as was present.

With regard to the tubular structure, the relative degree of vulnerability of the different tubules is found to correspond to that seen in acute toxic poisoning. The secreting tubules show early evidence of necrobiotic change, but no actual necrosis occurs; a fatty metamorphosis, which is preceded by a granular swelling of the protoplasm of the cell, is early and very constantly seen in the ascending looped tubules and convoluted tubules. In certain cases this would appear to be due to direct pressure from without, but in others it can only be due to the direct action of a toxin.

The loops of Henlé are most vulnerable, and there is evidence that obstruction of the blood supply to the glomerular unit plays little or no part in producing this change. In some of the acute cases tube casts of an albuminous nature are noticed.

The *interstitial tissue* shows, in the early cases, evidence of acute interstitial nephritis characterised by the accumulation of lymphocyte-

like cells, especially in the boundary zone, and radiating in zones around the interlobular vessels, and extending for a varying distance into the cortex. These cells are in the earlier stages almost entirely polyblasts and plasma cells; later, however, they have interspersed throughout them larger cells of endothelial and connective-tissue origin. They surround many of the glomeruli, and are interspersed through the tubules. At three days this appearance is evident (Plate XLIII. Fig. 4, Plate XLVI. Figs. 21 and 22). The formation of fibrous tissue takes place, following the lines of the interlobular vessels towards the surface. The creation of this is brought about by the organisation of the inflammatory exudate, of which the small cells form part, in the customary manner. It compresses the adjacent tubules and glomeruli. The characteristic feature is, however, well noticed in the manner, in which for a long time the process is entirely periglomerular, and only in its later stages does invasion occur, and fibroid glomeruli become produced, which may now be associated with tubular degeneration from an avascular cause; this, however, is not at all evident.

These are the main features of the changes as they are found in the kidney. I do not propose to discuss them in reference to the bearing they have upon the work that has been done on this subject. A few points may, however, be alluded to. The site of the primary interstitial lesion in this disease, as pointed out by Greenfield (1879), in contradistinction to that in the atrophic kidney, is found in the deeper part of the cortex, adjacent to the boundary zone. He also was the first to emphasise, in 1879, the further attention which the study of this disease then required. This investigation has also borne out another point referred to—the early reaction of the endothelium lining of Bowman's capsule, and the similarity of its behaviour to that of the endothelium adjacent to an inflammatory focus in the process of repair. The lymphoid nodes which have been so constantly described in this disease, and more especially by Councilman—in the acuter form of scarlatinal nephritis, are here also distinctive. The evidence would strongly bear out that, as this observer and others maintain, they consist (to a large extent) of plasma cells, under which category is included, by most observers, cells which Maximow would differentiate into polyblasts. These would appear to have emigrated from the blood stream and undergone a further metamorphosis. I have not, however, seen evidence of these locally increasing in numbers by cell division. There has not been seen in this investigation any evidence of these cells acting, to any appreciable extent, as phagocytes; their function here would, however, appear to be very similar to that which they fulfil in the former situations in which we found them.

The results of the above-described investigation were communicated to the Pathological Society of Great Britain and Ireland in July 1907. Since that date another animal has been killed. It was a young fox-terrier 10 months old when inoculated 4½ months prior to its

death. In it the tumours developed and then gradually disappeared. When killed the animal appeared in good health. The kidneys, however, showed advanced subacute interstitial nephritis similar to several already described. This last case, in my opinion, establishes the fact, beyond the possibility of question, that the renal changes were caused by the action of a virus produced by the infective sarcoma. This has also been further borne in upon me, as I have examined for the purpose of control kidneys of dogs which have died from various causes.

XII. The Relationship of Infective Sarcoma to Cancer.

As one would naturally expect to find from what has been seen of the nature of this disease, certain diversities of opinion exist in regard to the relationship it possesses to a true malignant neoplasm, such as sarcoma as it occurs in man. We have already seen how it possesses a histological structure which, in my opinion, is identical with that of a true alveolar sarcoma, and how this opinion is in conformity with that of virtually all those who have, more especially in the last few years, investigated the disease, with this reservation, that some would designate it a round-celled sarcoma and others a lymphosarcoma. Beebe and Ewing, in speaking of it, describe it as an infective lymphosarcoma, but come to the conclusion that the general histological characters suggest to them more the diagnosis of alveolar sarcoma or endothelioma, although they have failed to determine the particular tissue cell giving rise to the tumour. The main source of origin of the tumour cells formed in the tissues of the host after artificial inoculation we have seen to be from connective-tissue cells, although some undoubtedly arise from endothelial cells, and thus their latter conclusion has some evidence in support of it. My objections, however, to the use of the term endothelioma to describe the tumour from its histological appearance are, that this is not to any great extent the main source of origin of its cells, and the term endothelioma has, to my mind, become of late the Cave of Adullam, for the terminological designation of so many new growths. Those who have worked at this disease are fortunate in one respect, in that Sticker (1905) has been able to obtain specimens of the tumours, studied by Wehr, Geissler, Smith and Washbourn, Sanfelice, and Bashford, and, on submitting them with his own to a large number of German pathologists, found that they all were of the opinion that the lesions were identical, and of the nature of a round-celled sarcoma. This state of affairs would help to emphasise a point that is sometimes overlooked by some clinicians, that the diagnosis of a neoplasm is not always possible from the histological examination alone, and would also be evidence in favour of the importance of avoiding the divorce of pathology from clinical work.

Against it being a true malignant neoplasm the workers of the Imperial Cancer Research Laboratory advance the following arguments :—

1. *Its invariable infective history.*—This certainly is a point worthy of consideration; but in connection with it, although most pathologists will readily concede that the vast majority of those lesions classified under the generic term of cancer or malignant neoplasms do not infect, we have here one in which it is especially easy to trace the tumours formed to a definite source of contagion, from the nature of the act during which that occurs. This point is well illustrated by the comparison of the venereal and non-venereal warts, which are found growing from various epithelial surfaces of the human body. Cathcart has definitely proved that venereal warts, which are accepted by most pathologists as a variety of tumour, are contagious and independent of local irritation, although often associated with the presence of gonorrhœa; whereas, on the other hand, it has been extremely difficult to prove that non-venereal cutaneous warts are also, in certain cases at least, contagious. The case described by Payne (1891), in which he accidentally inoculated himself from a boy who had a copious eruption of these, proves conclusively that this can occur. Other illustrations might be cited to elaborate this point. Before, however, I leave it I may be allowed to quote the words used by Ohlmacher (1901) in speaking of the suspected origin of true lymphoma: "We cannot be too critical regarding this type of neoplasm, for if we are to eliminate those tumours in which an infective cause is suspected, and retain as true tumours only those in which an embryonic or non-infective, post-natal, causal influence can be proved, then it will appear in goodly company."

2. *The transformation of the surrounding connective-tissue cells into tumour cells*, even at the surface of fully developed tumours.—That this occurs in certain cases is, in my opinion, correct; but the limited extent to which it is present may well be realised when such competent observers as Sticker (working under the direction of such a master of pathological research as Ehrlich), and Beebe and Ewing, will not concede that it occurs. In answer to this criticism I would maintain that he who would claim to be able by microscopical examination of the histological structure of a fully developed sarcoma alone, to be so certain that infection of the surrounding connective-tissue cells never occurs, as to base upon this observation the tenet that the absence of infection was an invariable feature of all malignant neoplasms, is claiming for his powers of observation a degree of accuracy that few will concede to him.

3. *The process which occurs in artificial transmission* is identical with those by which tumour masses are formed as a result of inoculation with the tubercle or glanders bacillus. This has been discussed fully already. Points of resemblance do exist, and the deep underlying process is, in many respects, similar. The analogy is,

however, an unfortunate one, and it remains still to be proven, in what respects it differs from that which would occur in the creation of a true sarcoma.

4. *The disease never occurs naturally in animals before sexual maturity*, and is much less frequent in old age. One would naturally expect this from the source of infection—contagion during copulation.

5. The last point raised by them is the most important. *The process is definitely infective, in marked contrast to the changes which lead to the establishment of new tumours when a true malignant new growth is successfully transplanted.*

We have already seen how, in my opinion, the origin of the new tumour after inoculation is not entirely infective: but granted, what is probable, that this is the sole explanation of the origin of the sporadic tumours, one has certain criticisms to offer, as it raises a point of great importance in connection with the nature of true malignant neoplasms. It is referred to by Beebe and Ewing (1906) when they say, that all arguments in favour of this being a neoplasm prove fatally defective if, on transplantation, the tumour cells grow by infection of other cells. This is, in their opinion, the crucial test of an infective granuloma as against a neoplasm. Now, on what evidence is the contention based, that all malignant neoplasms grow from a single primary focus, have the power of apparent unlimited growth, and always graft when successfully inoculated; and what are the justifications for Fisch's remark when, in reviewing the progress of work on cancer research during the year that is past, supporting Bormann (1907) he says, "one of the main questions in the cancer problem is, to the impartial mind, settled? Tumours grow from the beginning through the multiplication of their own substance, destroy surrounding tissue in various ways, but never change it to tumour tissue."

The evidence in favour of the above contentions has been mainly obtained from the study of cancer by means of experimental investigation, in the course of which thousands of tumours have been inoculated into animals of the same and different species, with a result which has in virtually every case proved negative. In the carcinoma which occurs in mice, originally investigated and described by Jensen, this was not the case, as he was successful in inoculating it into other animals of the same species, and proved that the tumours formed were derived from the cells introduced. This tumour and others similar to it have been most carefully investigated by Ehrlich (1906), Bashford (1905), Borrel (1903), Gaylord (1906), and Clowes (1906), and through the generosity of Professor Jensen I have also been afforded the opportunity of studying its characters of experimental inoculation.

Briefly, the main facts concerning this tumour upon which the experimental investigation of cancer has been virtually entirely based in recent years are as follow: The tumour is one which occurs in

the subcutaneous tissue of mice. The discovery of a sporadic tumour is an event of rare occurrence, but when it is found it can, in contradistinction to other known malignant epithelial new growths, be readily inoculated into animals of the same species. Clinically and pathologically, this tumour is accepted by virtually all competent observers to be in all respects homologous with carcinoma as it occurs in man. It is looked upon by some as an adeno-carcinoma of the skin; by others as a mammary tumour. No evidence of spontaneous infection has been absolutely proven to occur, although Gaylord (1906) reports the occurrence of upwards of sixty sporadic tumours during three years, in animals kept in the same cage, the stock of animals being entirely renewed at least on one occasion, and Borrel has succeeded in tracing twenty sporadic tumours to animals all of which came from the same cage.

The percentage of successful inoculations varies. It is usually low at first, in succeeding generations this, however, rises, and may reach as high as 90 per cent. After introduction in a successful case there soon develops a tumour which grows rapidly, occasionally produces metastatic foci, and usually leads to the death of the animal. In a certian percentage of cases spontaneous recovery occurs, which renders the animal immune to subsequent inoculation. Ehrlich (1906) and Clowes (1906), who have studied the problem of this immunity, have found that they can render mice, which have not been successfully inoculated, immune against the disease by various methods. Ehrlich has also found that the nature of the tumours may be altered by repeated inoculations. In tumours of a rapidly growing strain he has been able by this means to make an adeno-carcinoma become a typical sarcoma, a change which he attributes to an irritative effect exerted by the cancer cells upon the cells of the stroma causing these mesodermic cells, whose proliferative activity has been so greatly augmented by repeated inoculations to grow luxuriantly and independently like cancer cells.

The parenchyma of the tumour which develops after inoculation was proved by Jensen to arise from the cells introduced, and this fact is now accepted by all.

The fate of the stroma introduced is, however, not considered by all to be the same.

Bashford says: "The stroma introduced at inoculation undergoes degeneration, and is replaced by supporting structures derived entirely from the tissue of the host."

Apolant and Ehrlich (1906), working with tumours similar in structure, but mostly of a more virulent strain, agree with the well known observation that "frequently in cancer transplantations the connective-tissue stroma inoculated with it undergoes atrophy, but the mesodermic cells of the tumours formed are derived from those of the sporadic growth."

Now, although I do not in any way desire to belittle the value of the facts concerning cancer, and the cancer process brought out by the study of the tumours in mice, I refuse to attach that importance to them which those would who appear to endeavour to make this mouse cancer the Mecca to which all devout pilgrims to the shrine of cancer research must journey for the true faith, demanding that whatever would claim to be cancer must conform in every particular to Jensen's tumour.

Especially would I refuse to grant that it is necessary for all sarcomata to conform to this standard in every respect before they can be accepted as true malignant neoplasms, when I find that the only reported cases of successful inoculation with sarcomata had a histogenesis identical with that described by me as occurring in this disease.

Von Eiselberg (1904–05), Velich (1898), and Loeb (1902) have all reported cases of successful inoculation with sarcomata. The last of these did a large number of experiments with a sarcoma of the white rat. He carried it through about forty generations. He also worked with other cases of sporadic sarcomata in the same animal, and obtained a similar facility in them for successful inoculation. The difficulties he encountered of determining the true nature of the process by which tumours were created were very much akin to those encountered by me in the dog. A mingling of cells derived from two sources occurred, and tumours were created by a grafting of cells of the portion introduced, along with a process of associated infection. In many other respects his tumours were similar to infective sarcoma as it occurs in the dog, and with it the vexed question also arose as to whether it was a true sarcoma or infective granuloma. In his opinion it was a true sarcoma. Under these circumstances I consider we are justified in withholding our opinion as to whether true sarcoma do or do not infect, and if the power the cancer cell is claimed to possess of apparent limitless propagation (which is the bedrock of the cytological school of cancer research) is disregarded, the comparison between the behaviour of Jensen's adeno-carcimona, as will have been already made out, and that of infective sarcoma is most striking,—both possess a histological structure identical to that of malignant neoplasms, and can be propagated with great facility by inoculation into animals of the same species, and by this means malignant neoplasms with secondary foci of the disease may be created. In both resorption and disappearance of the tumours may occur, and in each case this is followed by the establishment of immunity. In Jensen's tumour there is evidence in favour of the transference of the disease by contagion. In infective sarcoma this is the constant mode by which the disease is communicated.

If we look at some of the features we have noticed in the tumours we have examined some further evidence as to the nature of the disease may be obtained. In my opinion the strongest evidence so far brought forward in favour of its being an infective granuloma is

the establishment of the fact that in infective sarcoma a toxin is created which leads to the production of interstitial nephritis. No proof is forthcoming that in true sarcomata any such virus causing similar changes is created, although these naturally have been noticed to be present in some cases. This is a subject which, I would suggest, is worthy of investigation, as it is quite within the range of possibility that slight manifestations of renal inflammation might be detected, with a regularity which would warrant the assumption that they were caused by the presence of the neoplasm, and were not mere accidental coincidences. The cachexia which accompanies the development of true neoplasms is undoubtedly due in most cases to a contemporaneous toxic absorption from an associated ulceration or interference with alimentation; there are, however, cases in which it would appear to be due to the absorption of a poison produced by the new growth, but this has never been isolated or definitely proved to exist.

The presence of lymphocytes and their derivatives throughout the tumour, which was noticed at certain stages of its growth, and the accumulation of these cells forming extravascular accumulations beyond its extending border, occurs in true malignant neoplasms. This is found apart from any associated ulceration. In a true neoplasm this appearance is not uncommon, and I have seen it in an alveolar sarcoma removed by operation from a human being, which also illustrates the similarity of its histological structure to that which is seen in the dog. No satisfactory explanation of the presence of these cells in human sarcoma is so far forthcoming, and I am unable to find any record of a progressive lymphocytosis being a feature of such cases.

The spontaneous recovery which was found so frequently with tumours following subcutaneous inoculation is in accordance with that seen with Jensen's adeno-carcinoma. The ratio of these has varied materially in the experience of different workers. We have seen how, in the animals inoculated in the course of this investigation, it would probably have been high if they all had been allowed to live.

Sticker had eighteen recoveries out of sixty cases. The number of animals in which this occurs after natural infection is very much lower. It does, however, very occasionally take place. Spontaneous recovery after the development of a true malignant neoplasm in man, has been recorded by Orth (1904), Gaylord, and Clowes (6. 1906), and others, more especially with sarcomata, but it is difficult in these to exclude all possibility of fallacy.

The immunity which exists after the disappearance of the growths is in conformity with that found with the adeno-carcinoma of the mouse. The existence of this with true malignant neoplasms is, of course, incapable of demonstration; but it is in accordance with what one would expect to be present in the tissues of an organism, which has been able to overcome a similar previous infection.

The production of secondary foci of the disease by metastatic infection by way of the blood stream has been noticed in one of the cases. The secondary foci, however, did not show all the characteristics one would expect to see with the development of these from a single primary focus.

A consideration of the characteristics of infective sarcoma and a comparison of these with those of true malignant neoplasms, will show that it is a condition which in certain important features differs from the majority of malignant new growths. In no one prominent trait can it, however, be said to differ from all of those morbid processes which are at present included under the vague generic term of Cancer, Neoplasm, or New Growth. The differences are mainly in features which are assumed, but have not been proved to be absent in all true neoplasms. Thus the ease with which its origin can be traced to a definite process of contagion is a point in which it differs from the vast majority of the new growths met with in man. The fact that it is one where, combined with a local lesion, a toxin is created, and that we have been able to find definite inflammatory changes produced through the body, with other evidences of a general reaction, inclines one to the belief that we have here a disease which approximates in its nature to certain of the infective granulomata, such as syphilis. Its local lesion, however, resembles true sarcomata in histological structure and clinical characteristics. I would prefer, therefore, to describe it by the term by which it has been known, Infective Sarcoma, a venereal disease, and to consider it a tumour belonging to the borderland between the infective granulomata and the true neoplasms, one which probably in the near future will be added to the group of processes caused by a reaction of the tissues to a living virus, but one in which that virus is intra- and not extracellular, and the leading characteristic of which is the creation by this means of a structure resembling a true neoplastic new growth.

XIII. Final Conclusions.

The conclusions concerning the nature of infective sarcoma which have been arrived at as a result of this investigation are—

1. That infective sarcoma is a general disease characterised by the production of a local lesion possessing the histological structure of an alveolar sarcoma.

2. That it is caused by an intracellular virus conveyed by contagion from a living cell to a living cell.

3. That the tumours formed after natural infection probably consist of cells derived entirely from the tissues of the host.

4. That the nature of the virus cannot be detected, and (*a*) cannot be revealed by any method of staining; (*b*) does not pass through a Berkefeld filter; (*c*) is probably not an ultra-microscopical micro-

organism; (*d*) cannot be isolated apart from the tumour; (*e*) is not a spirochæte.

5. That the disease can be reproduced readily by subcutaneous inoculation into animals of the same species.

6. That by the same method it can be reproduced in the fox.

7. That the tumours following subcutaneous inoculation are created from the cells introduced; some of these live, and by a process of direct contagion lead to the formation of additional tumour cells from the connective-tissue cells of the host.

8. That it grows by multiplication of the cells thus formed, combined with a limited formation of additional tumour cells from the tissues of the host.

9. That this latter ceases with the development of the power of increased resistance on the part of the host, and a capsule is thus formed around the tumour.

10. That the tumours disappear by gradual cytolysis associated with the presence of, and probably caused by, lymphocytes and polyblasts.

11. That the growth of the tumour is accompanied by a gradual increase in the number of mononucleated cells in the circulation.

12. That immunity follows spontaneous recovery.

13. That this is brought about by a similar process occupying a much shorter duration of time—a cytolysis of the tumour cells accompanied by, and probably arising from, the presence of lymphocytes and polyblasts.

14. The growth of the tumour is associated with the development of a toxin which can be isolated from it by filtration and produces interstitial nephritis.

15. That the results of this investigation emphasise the present unsatisfactory classification of neoplasms which include a heterogeneous collection of pathological processes, and indicate the probability that, in the near future, a differentiation will be possible of processes that are really neoplastic from those that are merely reactive attempts by the host against an invading virus.

The experimental part of this research was conducted in the Laboratory of the Royal College of Physicians, to the President and Fellows of which College I desire to express my indebtedness for the opportunity thus afforded. The histological portion was carried out in the Laboratory of the Royal College of Surgeons; to the President and Fellows of that College I owe a similar debt of gratitude.

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DESCRIPTION OF PLATES.

Plate XLII.

Fig. 1.—Nodule removed after eighteen hours. Showing portion of tumour introduced and adjacent tissue of host. Intimate union between the two. Necrotic tumour tissue. The tumour formation area. The adjacent areolar tissue of the host in which the formation of additional tumour cells is occurring. (× 100, E.Mb.)

Fig. 2.—Nodule removed after eighteen hours. Showing the line of junction of the portion introduced and the tissue of the host. The tumour formation area, consisting of healthy tumour cells. Two in mitosis. Within, this degenerate tumour tissue invaded by leucocytes. On its outer aspect the margin of the adjacent areolar tissue with the formation of tumour cells from fibroblasts and endothelial cells. Polymorphonuclear leucocytes. Polyblasts. Fibrin. (× 600, P.R.Mg.)

Plate XLIII.

Fig. 3.—Disappearing nodule, one hundred and eighteen days. Showing the characteristic appearance in the centre of the nodule. Tumour cells swollen, vacuolated, and degenerated. Lymphocytes small dark. Polyblasts and plasma cells, bright red. (× 600, P.R.Mg.)

Fig. 4.—Kidney inoculation experiments, three days. Showing accumulations of lymphocyte-like cells radiating through the cortex along the course of the interlobular arteries, and infiltrating the adjacent interstitial tissues. (× 200, E.Mb.)

Plate XLIV.

Fig. 5.—Natural tumour, Case 1. Showing tumour covered by healthy vaginal epithelium. Invasion of the adjacent subcutaneous vessels. Extravascular accumulation of lymphocytes and polyblasts beyond the outer border of the tumour. (× 300.)

Fig. 6—Inoculation tumour. Second generation, first series. Showing structure of tumour. (× 300.)

Fig. 7.—Inoculation tumour. Second generation, first series. Secondary nodule in intestine. Showing nodule adherent to and invading muscular coat. (× 12.)

Fig. 8.—Inoculation tumour. Second generation, first series. Suprarenal gland. Showing tumour cells invading the suprarenal cortex. (× 300.)

Fig. 9.—Photo of inoculation tumour. Fifth generation, second series. Showing skin and subcutaneous tissue, with four nodules, two on section and two uncut.

Fig. 10.—Nodule in subcutaneous tissue, twenty-four hours after inoculation. (× 10.)

Plate XLV.

Fig. 11.—Inoculation tumour, eight days. Showing invasion of adjacent areolar tissue and the formation of additional tumour cells from this. (× 500.)

Fig. 12.—Inoculation tumour, fifteen days. Showing invasion of adjacent fibrous tissue tumour cells, extending along the extravascular planes (× 45.)

Fig. 13.—Inoculation tumour, twenty-two days. Showing structure of tumour and lymphatic capillary containing lymphocytes. (× 150.)

Fig. 14.—Inoculation tumour, twenty-two days. Showing formation of capsule with fibroblast at outer border of tumour in mitosis. (× 100.)

Fig. 15.—Inoculation tumour, disappearing nodules, one hundred and eighteen days. Showing situation and structure in subcutaneous tissue. (× 10.)

Fig. 16.—Inoculation tumour, disappearing nodules, one hundred and eighteen days. Showing laminated capsule, consisting of layers of fibrous tissue with islands of degenerating tumour cells, and polyblast between. (× 75.)

PLATE XLVI.

FIG. 17.—Kidney, filtration experiment, No. 1. Showing cells present in the interstitial tissue. (× 50.)

FIG. 18.—Kidney, filtration experiment, No. 1. Showing periglomerular change. (× 300.)

FIG. 19.—Kidney, filtration experiment, No. 2. Showing advanced interstitial nephritis. (× 40.)

FIG. 20.—Kidney, filtration experiment, No. 2. Showing formation of periglomerular fibrous tissue. Thickening and endothelial proliferation of Bowman's capsule. (× 300.)

FIG. 21.—Kidney, inoculation tumour, three days. Showing cells present in interstitial tissue, mainly polyblasts and lymphocytes. (× 400.)

FIG. 22.—Kidney, inoculation tumour, three days. Showing periglomerular changes. (× 200.)

Fig 1

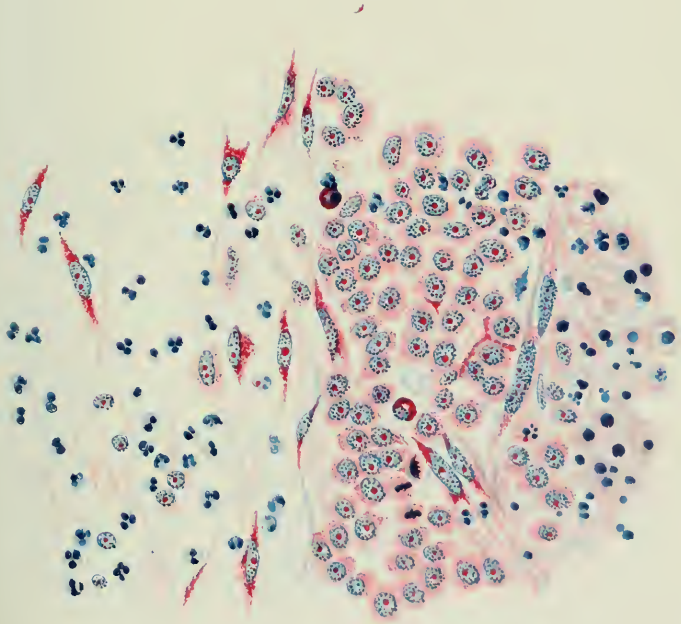


Fig .

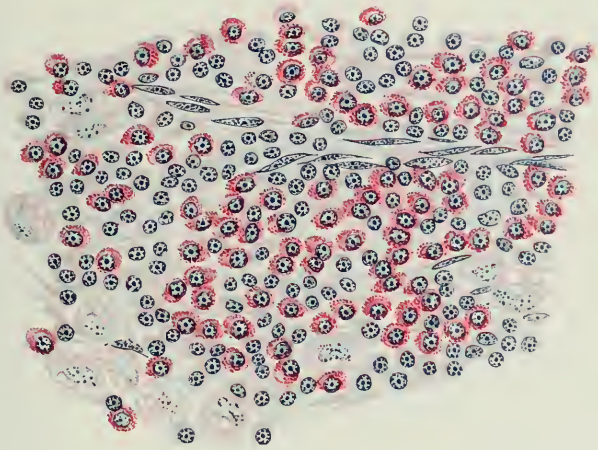


Fig 1

Fig 4

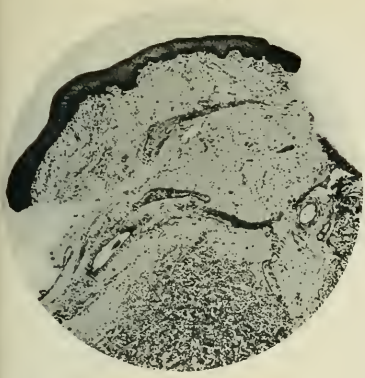
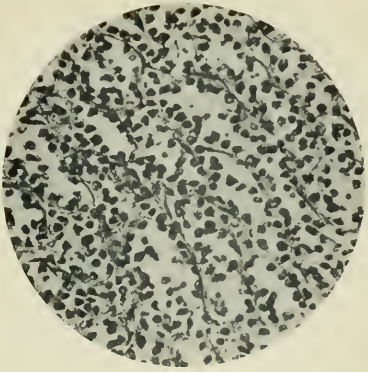


Fig. 5. (× 48.)



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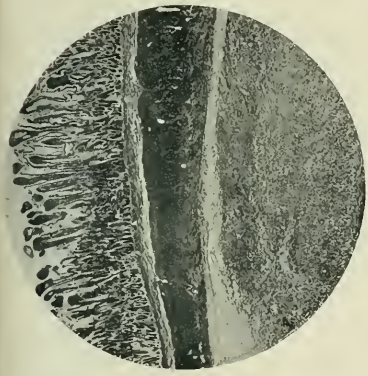

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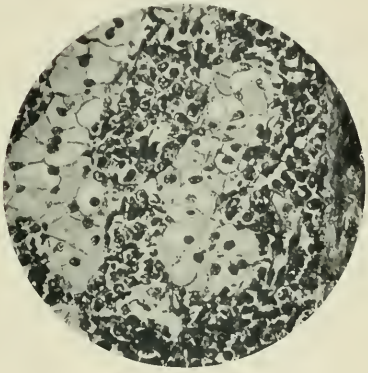

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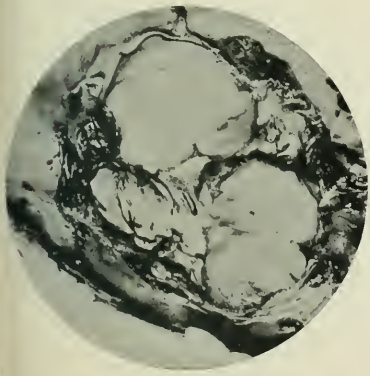
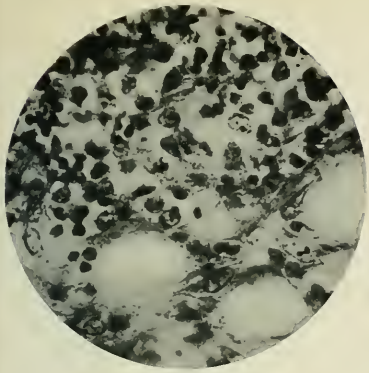


Fig. 9. (Nat. size.)

Fig 10. (× 10.)



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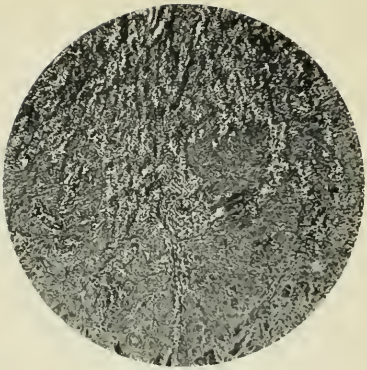

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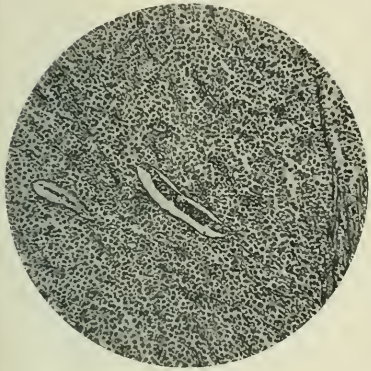
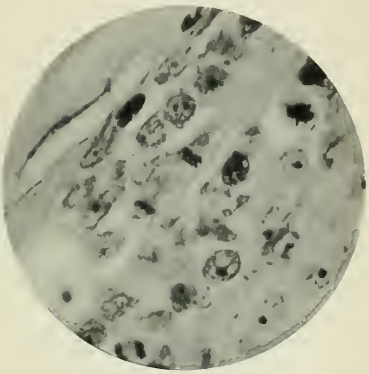


Fig. 13. (× 100.)



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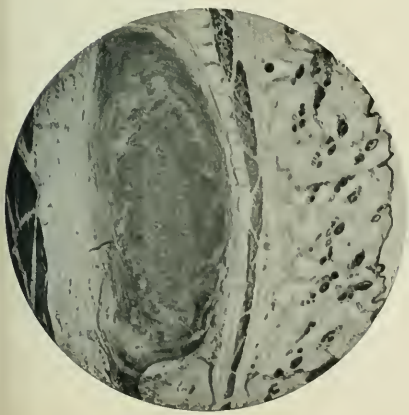

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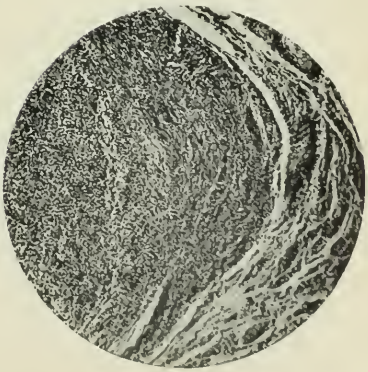

Fig. 16. (× 75.)

Fig. 17. (× 50.)

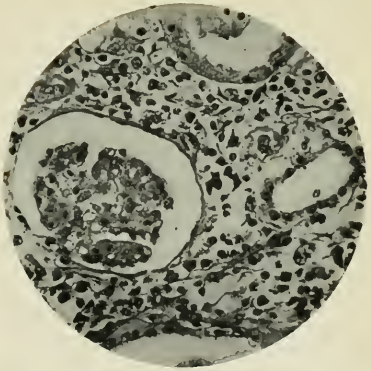

Fig. 18. (× 300.)

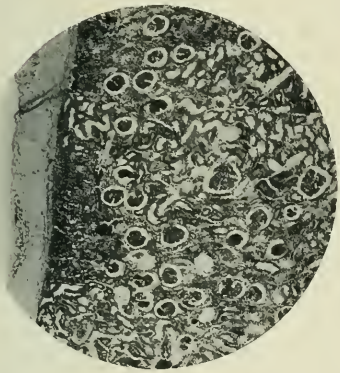

Fig. 19. (× 40.)

Fig. 20. (× 300.)

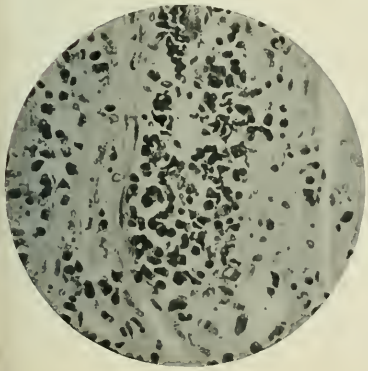

Fig. 21. (× 400.)

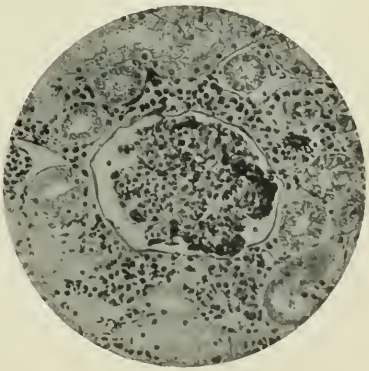

Fig. 22. (× 200.)

THE SPECIFICITY AND POTENCY OF ADRENOLYTIC AND THYMOLYTIC SERA.[1]

By W. T. Ritchie.

From the Laboratory of the Royal College of Physicians of Edinburgh.

A hæmolysin being a complex body, of which the two constituents are (*a*) a thermostable hæmolytic immune body, and (*b*) thermolabile complement, hæmolysis may be regarded as the manifestation of the toxic action of complement upon red blood corpuscles which have been sensitised by, or subjected to the influence of, the appropriate hæmolytic immune body.

Many investigations have been undertaken for the purpose of ascertaining whether analogous cytolytic sera can be obtained from animals which have been injected with other cellular elements—with ciliated epithelium (1), spermatozoa (2, 3, 4, 5, 6, 7), hepatic (8, 9, 10, 11, 12, 13, 14, 15, 16, 17), renal (9, 12, 14, 15, 16, 18, 19, 20, 21, 22, 23, 24, 25), and pancreatic cells (14, 26, 27, 28), or with the corresponding nucleo-proteids (11, 12, 13, 16), also with nerve tissue (29, 30, 31, 32, 33, 34), gastric mucous membrane (35, 36), placenta (37, 38, 39, 40, 41, 42), thyroid gland (43, 44, 45, 46), heart muscle (47), intestinal mucosa (57, 58), etc. While the earlier efforts to obtain potent cytolytic sera by the injection of tissue cells from the parenchyma of such organs as the liver and kidney appeared to be attended with no small measure of success, and while some recent writers (13, 16, 49, 51, 53) still affirm that the sera which they obtained were potent and specific, other investigators (16, 34) contend that whereas the serum of animals which have been treated with organs *in toto* is not strictly specific, yet animals treated with nucleo-proteids do yield a serum with a high degree of specificity. Most of the recent observations, however, and especially those of Czeczowiczka, 1903 ([18]), Pearce, 1904 ([14]), Woltmann, 1905 ([15]), and Pearce and Jackson, 1906 ([59]), indicate that "cytolytic" sera are not specific, and have little or no cytolytic action either *in vitro* or *in vivo*; their sole or chief action being a hæmolytic one.

I. Adrenolytic Serum.

The potency and specificity of adrenolytic serum have not hitherto engaged the attention of many investigators. In 1901 Bigart and Bernard ([31]) recorded the first observations on the subject. They found that the serum of ducks which had been injected with the adrenals of guinea-pigs was capable of killing these animals, the ratio

[1] Received October 9, 1907.

between the lethal dose of serum and the weight of guinea-pig being 1 : 125, and that the medullary portion of the adrenals of the guinea-pigs which succumbed was sometimes diffluent and gelatinous. These results have not been altogether substantiated by later observers.

Abbott's researches, 1903 ([50]), led him to doubt whether repeated injections of guinea-pigs' adrenals into rabbits result in the elaboration of a serum having demonstrable specific affinities for the adrenal glands of the guinea-pig *in situ*. Nor had the serum any cytolytic effect upon the cells of the guinea-pig's adrenals *in vitro*. It was hæmolytic, however, and its injection caused the death of guinea-pigs in five to ninety-two hours; but after the hæmolytic receptors had been removed from the serum it had no longer any toxic effect.

Sartirana, 1904 ([51]), found that the serum of hens which had been treated alternately with guinea-pigs' blood and adrenal extract was feebly cytotoxic and not hæmolytic. In other instances the serum obtained from the birds after their treatment with adrenals was found to have a toxic influence on the central nervous system. Yates, 1903 ([52]), concludes that "anti-adrenal" serum has little or no specific action.

The most important research is that of Pearce, 1904 ([14]), for he demonstrated that the activity of the serum differed according as to whether the animals had been treated with unwashed or with washed adrenals. In the former case the serum was toxic, a dose of 1 : 400 causing death in less than twenty-four hours. After doses of 1 : 900 to 1 : 300 the histological changes were indistinguishable from those due to a pure hæmolytic serum, or to a hepatolytic serum, and may all be readily explained by the action of hæmagglutinin or hæmolysin, but no changes referable to specific action on the adrenals could be detected. On the other hand, serum obtained from animals treated with washed adrenals agglutinated and hæmolysed red blood corpuscles *in vitro*, but hæmoglobinuria did not follow its injection. In doses of 1 : 700 to 1 : 550 this serum produced granular and fatty transformation of the liver and kidneys, but caused no lesion of the adrenal glands.

The results obtained by Pearce do not coincide with the observations of Levi della Vida, 1904 ([53]), who found that the serum of five ducks which had been treated with guinea-pigs' adrenals was not positively active after injection. A dose of serum equivalent to 2·5 per cent. of the weight of the guinea-pig failed to produce any disturbance. Levi della Vida, however, maintains that the serum was undoubtedly cytolytic *in vitro*, and as a result of his tests he upholds the specificity of action *in vitro* of the sera of animals treated respectively with the cortical and medullary portions of adrenal glands. The sera were hæmolytic as well as adrenolytic, and were rendered inert by being heated to 55° C.

Foà, 1905 ([54]), made many attempts to prepare adrenolytic sera in rabbits, guinea-pigs, and ducks, but his results were uniformly negative; while Stradiotti, 1905 ([55]), records a fall of blood pressure, to the extent of 25 mm. of mercury after the intravenous injection of the serum of dogs previously treated with adrenal extract.

The chief aim of our research was to determine (*a*) whether it be possible to prepare a serum containing an adrenophilic immune body; (*b*) whether the affinity of this immune body be specific; and (*c*) whether serum containing adrenophilic immune body and complement be adrenolytic.

As the sera employed in our experiments were obtained from ducks which had been repeatedly injected with adrenal glands of

guinea-pigs it is necessary to consider briefly the effect of normal duck's serum on the guinea-pig, and its action on the adrenal gland in particular.

A series of tests was first made in order to prove that the fresh normal duck's serum with which we were working contained complement, which in the presence of the appropriate immune body hæmolysed the red blood corpuscles of the ox—

Ox R. + H.I.B. + Normal duck's serum = Hæmolysis.

A second series of tests demonstrated that fresh normal duck's serum also contains a cytophilic immune body which permits the fixation of duck's complement to the molecules of the guinea-pig's tissue cells.

The presence of this cytophilic immune body was determined in the manner devised by Bordet and Gengou, 1901([56]). To three tubes, containing a finely divided portion of guinea-pigs' liver,[1] spleen, and adrenal respectively, 0·1 c.c. of fresh normal duck's serum (N.D. serum) was added, and after the mixtures had been left for three and three-quarter hours at 37° C. 1 c.c. of a 5 per cent. suspension of ox red blood corpuscles in normal saline solution (Ox R.), and 0·1 c.c. of heated serum (H.I.B.) containing a hæmolytic immune body for the red blood corpuscles of the ox (the serum of a rabbit previously injected with whipped ox blood and heated to 57° C. for one hour in order to inactivate the complement) were added to each. The tubes were then replaced within the incubator, but in no instance did hæmolysis occur, as is shown in the following table:—

		Hours at 37° C.	
Guinea-pig's liver	+ N.D. serum	$3\frac{3}{4}$	+ Ox R. + H.I.B. = No hæmolysis
,, spleen	+ ,,	,,	+ ,, + ,, = ,,
,, adrenal	+ ,,	,,	+ ,, + ,, = ,,

Now, if duck's complement possessing a zymotoxic atom group may be fixed to the guinea-pig's tissue cells, it might be supposed that normal duck's serum would exert a cytolytic influence upon those cells, and thus induce toxic symptoms, or even possibly cause the death of the animal receiving the serum.

In conformity with the results recorded by Bigart and Bernard, 1901([49]), Levi della Vida, 1904 ([53]), and other investigators, however, it was found that normal duck's serum is very feebly if at all toxic for the guinea-pig. A guinea-pig of 362 grms. received 3 c.c. of normal duck's serum intraperitoneally (0·82 per cent. of body weight), and yet a fortnight later was still alive and well. A similar negative result followed the intraperitoneal injection of 4 c.c. of normal duck's serum into a guinea-pig weighing 195 grms.

The tests recorded above prove that in the case of normal duck's

[1] All the guinea-pigs' organs used in this and in subsequent tests *in vitro* were obtained from animals which, having been killed by chloroform anæsthesia, were washed out with sterilised saline solution through a cannula tied into the aorta.

serum the absence of toxic action upon the guinea-pig cannot be explained by any deficiency in haptophore or zymotoxic atom groups on the part of the complement. The impotency of normal duck's serum upon the guinea-pig must therefore be due either to non-affinity of the zymotoxic atom group of the complement for the toxophile atom group of the guinea-pig's tissue cells, or to deficiency of immune body in the serum, so that sufficient complement to induce cytolysis cannot be fixed to those cells. If the zymotoxic atom group of duck's complement have little or no affinity for the toxophile atom group of the guinea-pig's tissue cells the addition of an appropriate cytophilic immune body will fail to render the serum cytolytic, whereas if the impotency of normal duck's serum be due solely to deficiency of cytophilic immune bodies cytolysis might perhaps be induced by the employment of a serum to which a sufficiency of an appropriate immune body had been added.

In the hope of obtaining such an immune body a series of ducks were repeatedly treated, by intraperitoneal injection, with suspensions of guinea-pigs' adrenal glands in normal saline solution. The glands were obtained with aseptic precautions from guinea-pigs which had been bled and killed under ether anæsthesia. In some instances the ducks developed a transient paresis of one leg shortly after each injection, but as a rule the birds withstood the injections well, and gained weight during the periods over which the injections were being given. Four of the birds were eventually, while under ether anæsthesia, bled from the carotid artery, and the serum was either used for tests *in vitro*, or was injected intraperitoneally into guinea-pigs.

Before considering the action of the treated ducks' serum reference must be made to two control observations—(1) that the normal guinea-pigs' serum which was employed in the subsequent tests did not contain a hæmolytic immune body for the red blood corpuscles of the ox, but did contain complement which in the presence of the appropriate immune body hæmolysed those corpuscles; and (2) that normal guinea-pigs' serum (N. Gp. serum) does not contain an immune body capable of fixing guinea-pig's complement to the adrenal cells of the guinea-pig. The latter point was determined by the following tests *in vitro*:—

	Hours at 37° C.	
Gp. adrenal + N. Gp. serum (0·10 c.c.)	1	+ Ox R. + H.I.B. (0·20 c.c) = Hæmolysis
,, + ,, (0·15 ,,)	2	+ ,, + ,, (0·10 ,,) = ,,
,, + ,, (0·15 ,,)	3	+ ,, + ,, (0·05 ,,) = ,,

We now pass to the consideration of the serum of the ducks which had been treated with guinea-pigs' adrenals, and find—

I. That the serum contained an adrenophilic immune body, in the presence of which guinea-pig's complement became fixed to guinea-pigs' adrenals. This was demonstrated by the following tests, in which "T.D. Serum" signifies the serum of a duck which had been injected with guinea-pigs' adrenals:—

	Hours at 37° C.	
Gp. adrenal + T D. serum (0·1 c.c.) + N. Gp. serum (0·1 c.c.)	4⅓	+ Ox R. + H.I.B. (0·05 c.c.) = No hæmolysis
Gp. adrenal + T.D. serum (0·1 c.c.) + N. Gp. serum (C. 15 c.c.)	,,	+ Ox R. + H.I.B. (0·05 c.c.) = No hæmolysis

A control test proved that the guinea-pig's serum which was employed really contained complement—

	Hours at 37° C.	
Gp. adrenal + N. Gp. serum . .	4⅓	+ Ox R. + H.I.B. = Hæmolysis

The tests here recorded indicate that there was no free complement in the mixtures of adrenal, treated duck's serum, and normal guinea-pig's serum at the time when the red blood corpuscles and hæmolytic immune body were added. It has already been shown that the fixation of complement to the guinea-pig's adrenal cannot be ascribed to the presence of an adrenophilic immune body in guinea-pigs' serum, and consequently the fixation must have been brought about by the presence of an immune body in the serum of the treated duck. As it has an affinity for the receptors of the guinea-pig's adrenal cells, this immune body may be termed an adrenophilic immune body, and the serum containing it an adrenophilic serum.

II. In order to test the specificity of this immune body, adrenophilic serum (obtained from a duck previously injected with guinea-pigs' adrenals) and normal guinea-pig's serum were added to a series of tubes containing respectively suspensions of guinea-pig's adrenal, spleen, kidney, etc., in normal saline solution. The tubes were then left for 4⅓ hours at 37° C., and thereafter 1 c.c. of a 5 per cent. suspension of whipped ox blood in normal saline solution and 0·05 c.c. of hæmolytic immune body were added to each, and the tubes replaced in the incubator for one hour. The presence or absence of free complement in each tube, and therefore the affinity of the adrenophilic

immune body for the receptors of the various cells of the guinea-pig, were thus determined :—

	Hours at 37° C.						
(1) Gp. adrenal + Adrenophilic serum (0·1 c.c.) + N. Gp. serum (0·1 c.c.)	1½	+	Ox R.	+	H. I. B. (0·05 c.c.)	=	No hæmolysis after 1 hr. at 37° C.
(2) Gp. adrenal + Adrenophilic serum (0·1 c.c.) + N. Gp. serum (0·15 c.c.)	,,	+	,,	+	,,	=	,,
(3) Gp. spleen + Adrenophilic serum (0·1 c.c.) + N. Gp. serum (0·1 c.c.)	,,	+	,,	+	,,	=	Slight hæmolysis after 1 hr. at 37° C.
(4) Gp. kidney + Adrenophilic serum (0·1 c.c.) + N. Gp. serum (0·1 c.c.)	,,	+	,,	+	,,	=	,,
(5) Gp. liver + Adrenophilic serum (0·1 c.c.) + N. Gp. serum (0·1 c.c.)	,,	+	,,	+	,,	=	Marked hæmolysis after 1 hr. at 37° C.
(6) Gp. lung + Adrenophilic serum (0·1 c.c.) + N. Gp serum (0·1 c.c.)	,,	+	,,	+	,,	=	,,
(7) Gp. muscle + Adrenophilic serum (0·1 c.c.) + N. Gp. serum (0·1 c.c.)	,,	+	,,	+	,,	=	,,
(8) Gp. heart muscle + Adrenophilic serum (0·1 c.c.) + N. Gp. serum (0·1 c.c.)	,,	+	,,	+	,,	=	,,
(9) Gp. thymus + Adrenophilics erum (0·1 c.c.) + N. Gp. serum (0·1 c.c.)	,,	+	,,	+	,,	=	,,
(10) Gp. brain + Adrenophilic serum (0·1 c.c.) + N Gp. serum (0·1 c.c.)	,,	+	,,	+	,,	=	,,
(11) Gp. spinal cord + Adrenophilic serum (0·1 c.c.) + N. Gp. serum (0·1 c.c.)	,,	+	,,	+	,,	=	,,
(12) Gp. bone marrow + Adrenophilic serum (0·1 c.c.) + N. Gp. serum (0·1 c.c.)	,,	+	,,	+	,,	=	,,

Whereas tubes 1 and 2 contained no free complement in virtue of the affinity of the adrenophilic immune body for the receptors of the guinea-pig's adrenal cells, there was either slight or well-marked hæmolysis in all the other tubes. These had therefore contained free complement at the time when the red blood corpuscles and the hæmolytic immune body were added. The incomplete hæmolysis in tubes 3 and 4 indicates that there had been a partial fixation of complement to the cells of the guinea-pig's spleen and kidney, and consequently a certain degree of affinity between the receptors of those cells and the adrenophilic immune body. But as there was no evidence of fixation of complement to the guinea-pig's liver, brain, thymus, etc., the inference is that this immune body had either no affinity or only a very slight one for the receptors of the cells in question. It is thus evident that the adrenophilic immune body has a greater affinity for the receptors of adrenal cells than for those of

other tissue cells, but it has a slight affinity for those of the spleen and kidney, and is therefore not strictly specific.

III. The hæmolytic activity of various cytolytic sera is admitted by many of those who have studied the subject.

Woltmann, 1905 ([15]), is of opinion that all the pathological changes discernible in the organs of animals which have been injected with cytolytic sera may be regarded as the direct or indirect effects of hæmolysis. As regards adrenolytic serum, its hæmolytic activity has been admitted by Abbott, 1903 ([50]), and by Levi della Vida, 1904 ([53]). Our observations, however, appear to substantiate those of Sartirana, 1904 ([51]), and of Pearce, 1904 ([14]), for according to the latter observer the serum of animals treated with washed adrenals was less actively hæmolytic than that of animals treated with unwashed adrenals, and the ducks furnishing the serum for our tests were injected with adrenals obtained from animals which had been thoroughly bled.

In such serum we failed to demonstrate any appreciable affinity on the part of the adrenophilic immune body for the receptors of guinea-pig's red blood corpuscles. In the following tests "Gp. R." signifies a suspension of guinea-pig's blood in normal saline solution, and "Gp. C." normal guinea-pig's serum containing complement, while the other abbreviations have the same significance as before :—

	Hours at 37° C.	
Gp. R.	3	= No hæmolysis
,, + Gp. C. (0·15 c.c)	3	= ,,
,, + Adreno serum (0·1 c.c) + ,,	3	= ,,
Ox R. + H.I.B. (0·1 c.c.) + ,,	½	= Complete hæmolysis

That the adrenophilic immune body had no special affinity for the receptors of the guinea-pig's red blood corpuscles was also shown by the absence of any structural changes referable to hæmolysis in the organs of guinea-pigs which had been injected with duck's serum containing adrenophilic immune body and complement.

IV. The stability of the immune body was determined by the following tests :—

	Hours at 37° C.	
1. Gp. adrenal	4½	+ Ox R. + H.I.B. = No hæmolysis
2. ,, + Adreno serum (8 days old) + Gp. C.	2	+ ,, + H.I.B. = No hæmolysis
3. ,, + ,, (20 ,,) + ,,	3¾	+ ,, + H.I.B. = Faint hæmolysis
4. ,, + ,,	3¼	+ ,, H.I.B. = Marked hæmolysis

Comparing the results of tests 2 and 3 it is evident that the affinity of the adrenophilic immune body in serum twenty days old was appreciably less than in the same serum when only eight days old; but the lesser degree of hæmolysis in test 3, as compared with that in test 4, indicates that the immune body to a certain extent still retained its affinity in serum twenty days old.

V. The adrenophilic immune body was thermostable at 51° C., but not at 57° C. The thermostability of the immune body at 51° C. was shown by the next test:—

	Hours at 37° C.	
Gp. adrenal + Adreno serum heated to 51° C. for 1 hour (0·1 c.c.) + Gp. C. (0·15 c.c.)	2½	+ Ox R. + H.I.B. (0·1 c.c.) = No hæmolysis

That the absence of hæmolysis in the above test was really due to fixation of complement, and was not due to deficiency either of complement in the guinea-pig's serum employed or of hæmolytic immune body, was proved by two control tests:—

Ox R. + Gp. serum (0·15 c.c.) = No hæmolysis	+ H.I.B. (0·1 c.c.) = Hæmolysis
,, + H.I.B. (0·1 c.c.) = ,,	...

The following tests indicated that the adrenophilic immune body was not thermostable at 57° C.:—

		Hours at 37° C.	
1.	Gp. adrenal + Adreno serum (heated to 57° C. for 1 hr.) + Gp. C.	2	+ Ox R. + H.I.B. = Hæmolysis
2.	,, + ,, + ,,	3	+ ,, − .. = ,,
3.	,, + ,, + ..	3¾	+ ,, − .. = ..
4.	,, + ,,	2	+ .. − ,, = ..
5.	,, + ,,	2	+ .. .. = No hæmolysis

Tests 4 and 5 of the above series are controls. As we were unaware of any similar instability at 57° C. having been recorded in the case of other cytolytic immune bodies, it was thought that our results might possibly be ascribed to some error in technique, but when the tests were subsequently repeated with the heated adrenophilic serum of another treated duck the immune body was again found to be thermolabile.

VI. In order to test its adrenolytic power, the serum of the treated ducks was injected intraperitoneally into seven guinea-pigs.

One received an injection of serum equal to 0·65 per cent. of its body weight; thirty-one days later the animal was still quite well, and was then given a second injection, equal to 1·1 per cent. of its body weight. Four and a half hours later the guinea-pig died. No evidence of peritoneal infection was detected, and all the organs, including the adrenals, were healthy, both the cortical and the medullary cells of these glands being in every respect unaltered.

The negative effect of the serum in the remaining six guinea-pigs is illustrated by the following table :—

Guinea-pig	Weight.	Dose of Serum in relation to Body Weight.	Result.
	Grms.		
2	500	0·25 per cent. . .	Well three months later; then lost weight, and died in six months
3	344	0·58 ,, . .	Well forty-six days later; then given 0·26 per cent. of the serum; died eighty-seven days later
4	329	0·66 ,, . .	Well forty-six days later; then given 0·83 per cent. of the serum; died ninety-seven days later
5	370	0·80 ,, . .	Well forty-six days later; then given 0·76 per cent. of the serum; died five months later
6	300	1·30 ,, . .	Died thirteen days later; tuberculosis
7	145	1·40 ,, +0·6 per cent. of normal duck's serum	Well three months later

These results differ from those of Bigart and Bernard, 1901([49]), but are in essential agreement with the observations recorded by Sartirana, 1904 ([51]), Yates, 1903 ([52]), Pearce, 1904 ([14]), Levi della Vida, 1904 ([53]), and Foà, 1905 ([54]).

Summary

1. The serum obtained from ducks which had been treated with guinea-pigs' adrenals contained an adrenophilic immune body.

2. In the presence of this immune body guinea-pig's complement became fixed to guinea-pigs' adrenals.

3. The serum was not strictly specific, but there was no evidence of it being hæmolytic.

4. The adrenophilic immune body was thermostable at 51° C., but not at 57° C.

5. The serum, although adrenophilic, was not adrenolytic.

6. The absence of adrenolytic action is probably due to the zymotoxic atom group of duck's and guinea-pig's complement having little or no affinity for the toxophile atom group of guinea-pigs' adrenals.

II. Thymolytic Serum.

In the following experiments an attempt was made to obtain a thymolytic serum from ducks which had been treated with the thymus glands of guinea-pigs.

Three ducks received intraperitoneal injections of suspensions of thymus glands in normal saline solution. Duck 1 was given four injections, at intervals of ten days, and received eleven and a half thymus glands in all. The average weight of the guinea-pigs furnishing the glands was 338 grms., the lightest being 210 grms., the heaviest 505 grms. Duck 2 similarly received fourteen and a half thymus glands, and duck 3 received thirteen glands.

The following tests demonstrated that the serum of the treated ducks contained an immune body having an affinity for receptors of the guinea-pig's thymus and lymphatic glands, spleen, and bone marrow, and that in the presence of this immune body guinea-pig's complement became fixed thereto, but that the immune body had no affinity for receptors of the guinea-pig's liver, adrenal, thyroid, kidney, etc. :—

[*Note.*—Where it is indicated below that thymus serum was used the amount added was 0·1 c.c.]		Hours at 37° C.			
Gp. thymus	+ Gp. C. (0·1 c.c.)	$1\frac{3}{4}$	+ Ox R. + H.I.B. (0·02 c.c.)		= Hæmolysis
,, ,,	+ Thymus serum + Gp. C.	,,	,,	+ ,, + ,,	= No hæmolysis
,, lymph gland	+ Thymus serum + Gp. C.	,,	,,	+ ,, + ,,	= ,,
,, ,,	+ Gp. C.	,,	,,	+ ,, + ,,	= Hæmolysis
,, bone marrow	+ Thymus serum + Gp C.	,,	,,	+ ,, + ,,	= Very slight hæmolysis
,, ,,	+ Gp. C.	,,	,,	+ ,, + ,,	= Hæmolysis.
,, liver	+ Thymus serum + Gp. C.	,,	,,	+ ,, + ,,	= ,,
,, lung	+ Thymus serum + Gp. C.	,,	,,	+ ,, + ,,	= ,,
,, adrenal	+ Thymus serum + Gp. C.	,,	,,	+ ,, + ,,	= ,,
,, thyroid	+ Thymus serum + Gp. C.	,,	,,	+ ,, + ,,	= ,,
,, kidney	+ Thymus serum + Gp. C.	,,	,,	+ ,, + ,,	= ,,
,, red blood corpuscles	+ Thymus serum + Gp. C.	,,	,,		= No hæmolysis

The combining affinities of the immune body indicated that we were dealing with a leucophilic immune body, and not with a specifically thymophilic one. The serum of the treated ducks had no

hæmolytic action, and moreover tests *in vitro* demonstrated that the immune body had no affinity for the receptors of the kitten's spleen, thymus, lymphatic gland, or bone marrow. That the leucophilic immune body was thermostable was ascertained by the following tests, in which "H. Thymus Serum" designates treated duck's serum that had been heated to 57° C. for one hour :—

[*Note.*—In the following tests the amount of heated thymus serum and of Gp. serum was 0·1 c.c.]	Hours at 37° C.	After thirty-five Minutes at 37° C.
Gp. thymus + H. Thymus serum + Gp. C.	5 hrs.	+ Ox R. + H.I.B. (0·025 c.c.) = No hæmolysis
,, ,, ,,	,,	+ ,, + ,, = Marked hæmolysis
,, lymph-gland ,,	,,	+ ,, + ,, = Hæmolysis
,, ,, + H. Thymus serum ,,	,,	+ ,, + ,, = No hæmolysis
,, spleen ,,	,,	+ ,, + ,, = Hæmolysis
,, ,, + H. Thymus serum ,,	,,	+ ,, + ,, = No hæmolysis
,, bone marrow ,,	,,	+ ,, + ,, = Hæmolysis
,, ,, + H. Thymus serum ,,	,,	+ ,, + ,, = No hæmolysis
,, adrenal + H. Thymus serum ,,	,,	+ ,, + ,, = Slight molysis
,, ,, ,,	,,	+ ,, + ,, = Hæmolysis
,, liver + H. Thymus serum ,,	,,	+ ,, + ,, = ,,
,, kidney + H. Thymus serum ,,	,,	+ ,, + ,, = ,,
,, ,, ,,	,,	+ ,, + ,, = ,,
,, muscle + H. Thymus serum ,,	,,	+ ,, + ,, = ,,
,, lung + H. Thymus serum ,,	,,	+ ,, + ,, = ,,
,, pancreas + H. Thymus serum ,,	,,	+ ,, + ,, = ,,

Having thus ascertained that it contained a thermostable leucophilic immune body, five guinea-pigs were injected with the serum. The first three were given unheated serum, the remaining two received serum which had been heated for one hour at 57° C. on two occasions.

Guinea-pig.	Weight.	Dose of Serum in relation to Body Weight.	Injection given.	Result.
1	220 grms.	1·8 per cent.	Intraperitoneally	Died on third day
2	110 ,,	1·8 ,,	,,	Died in nineteen hours
3	156 ,,	1·6 ,,	Subcutaneously	Remained well
4	160 ,,	2·0 ,,	Intraperitoneally	,,
5	135 ,,	2·0 ,, + 1·8 per cent. of normal guinea-pig's serum.	,,	,,

Our tests *in vitro* having demonstrated that the serum contained a leucophilic immune body, it was to be expected that the serum would have a leucolytic action. But Moorhead, 1905 ([60]), found that the serum of rabbits which had been injected with guinea-pigs' thymus glands had no recognisable leucolytic action, did not agglutinate emulsified thymus gland *in vitro*, and had no constant effect upon the animals into which it was injected. The serum we employed was undoubtedly leucolytic. Injection of the serum into guinea-pigs occasioned a leucocytosis, as, for example, in guinea-pig No. 4, where the leucocytes numbered 2400 per c.mm. on the day before injection, and 10,400 per. c.mm. on the day after it. In guinea-pig No. 5 the leucocyte count was 6500 per c.mm. on the day after injection, and 22,000 per c.mm. on the succeeding day.

In only one instance was it possible to say that the serum had acted specially upon the thymus gland. In guinea-pig No. 1, which weighed 220 grms. and died on the third day after being injected, the lobules of the thymus gland were small, the differentiation between the cortical and medullary portions of each lobule was not well marked, and the width of the cortical portion was much reduced, so that some of the Hassal's corpuscles, which in this and all other cases were themselves normal, lay at the extreme periphery of the lobule. This change is manifestly evidence of leucolysis, and yet neither the bone marrow nor the lymphatic glands showed any changes referable to the action of a leucolytic serum.[1]

Summary.

1. The serum obtained from ducks which had been treated with the thymus glands of guinea-pigs contained a leucophilic immune body.

2. In the presence of this immune body guinea-pig's complement became fixed to guinea-pig's thymus, lymphatic gland, bone marrow, and spleen.

3. The serum was not hæmolytic.

4. The leucophilic immune body was thermostable.

5. The structural changes in the thymus of the guinea-pig following injection of the serum were due to its leucolytic action, and are not specific.

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IN VIVO EXPERIMENTS WITH COMPLEMENT.

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The following experiments were undertaken with the object of determining what change, if any, take place in the complement content of the serum of fresh and immunised rabbits (*v.* ox blood corpuscles) on the intravenous injection of unsensitised, sensitised,[2] and saturated ox blood corpuscles. Rabbits were bled from the ear vein to the amount of 15 c.c.; they were then injected with the ox blood corpuscles, and usually in one and a half hours, sometimes after an hour, blood was again obtained from them either from the ear vein or from the internal jugular. These samples were allowed to clot, and the serum of each was allowed to remain in contact with the clot for the same period of time, to have the conditions as nearly as possible comparable; and, as far as circumstances would allow, everything was done that was essential for a strict comparison of the sera before and after injection. What was aimed at in regard to the dosage of corpuscles was to give a dose sufficient to use up all the complement present in the animal's body; it was considered inexpedient to experimentally determine the amount of complement present in the animal to be used as a guide to the dose of corpuscles to be given, as this would have involved such an exhaustive bleeding of the animal, previous to the bleeding at the time of the experiment, as was bound to have an effect on the economy of the animal. It was thought that it would serve the purposes of the research if a dose of corpuscles, in round numbers judged to be about equal to that necessary to use up all the complement in the animal's body, was given. As the amount of complement varies in the serum of different rabbits, this dose was afterwards seen sometimes to be less than the actual amount ascertained by subsequent calculations, sometimes it was greater. In calculating after the experiment how much of the complement—supposing that the complement circulated free in the plasma—ought theoretically to have been used up in the experiment, the M.H.D. of

[1] The subject of a communication to the Pathological Society of Great Britain and Ireland, January 3–4, 1908. [Received for publication February 18, 1908.]

[2] Corpuscles can take up several multiples of the animal hæmolytic dose or sensitising dose; when corpuscles are treated with these multiples they are said to be saturated.

complement estimated in the serum of the animal "before injection" was used. The amount of blood in the animal's body was calculated from Douglas' (1905–6) figures, which give 4·85 per cent. of body weight of blood in the buck and 5·3 per cent. of body weight in the doe. Rabbit serum amounts to about 50 per cent. of the blood. This was determined by taking small tubes with 3 c.c. of blood and centrifugalising them with a hand centrifuge at 4000 revolutions per minute, until the clot was deposited as far as it would go. From these data it could be estimated how much of the total complement of the animal's body—supposing that complement was a limited definite quantity circulating in the plasma—ought to have been used up by the dose of ox blood corpuscles injected.

EXPERIMENTS.

Series I.

To determine the change in complement content of the serum of a fresh rabbit after injection intravenously of *sensitised* ox blood corpuscles.

Experiment 1.

Rabbit, buck; weight, 2000 grms.; ox blood corpuscles were washed 6 times; and the M.H.D. of the heated immune body used to sensitise them was 0·012 c.c. of a 1 in 6 dilution for 1 c.c. 1 per cent. suspension. The rabbit was bled to the extent of 15 c.c., then immediately a mixture of 2 c.c. ox corpuscles + 2 c.c. 0·85 per cent. saline + 0·4 c.c. heated immune serum (sensitising dose calculated from the M.H.D. given above) was injected into the ear vein. This mixture had been kept at 37° C. for 1½ hour previous to injection. The animal showed no symptoms of distress after injection, and survived. One and a quarter hour after injection 15 c.c. of blood were again drawn from the ear vein. The sera "before" and "after" were allowed to separate, each remaining in contact with the clot for the same time. The sera were then decanted and the estimation of the complement undertaken. (To avoid repetition it may be said that the same routine was gone through in the other experiments.) The serum "after" injection was deeply hæmoglobin tinted.

Determination of Complement.

Complement was diluted 1 in 3 with saline (0·85 per cent.); heated immune serum was diluted 1 in 6 with saline 0·85 per cent.; M.H.D. for 1 c.c. 1 per cent. suspension was 0·012 c.c.; 1 per cent. suspension of ox blood corpuscles used.

Before Injection.

	1 per cent. Suspension O.B.C.		H.I.S.		Compt.		0·85 per cent. Saline.		
(1)	1 c.c.	+	0·012	+	0·6	+	0	=	+ + + +[1]
(2)	1 ,,	+	0·012	+	0·55	+	0·05	=	+ + + +
(3)	1 ,,	+	0·012	+	0·5	+	0·1	=	+ + + +
(4)	1 ,,	+	0·012	+	0·45	+	0·15	=	+ + + +
(5)	1 ,,	+	0·012	+	0·4	+	0·2	=	+ +
(6)	1 ,,	+	0·012	+	0·35	+	0·25	=	+
(7)	1 ,,	+	0·012	+	0	+	0·6	=	0

[1] + + + + = nearly complete hæmolysis.

After Injection.

	1 per cent. Suspension O.B.C.		H.I.S.		Compt.		0·85 per cent. Saline.		
(1)	1 c.c.	+	0·012	+	0·6	+	0	=	+ + + +
(2)	1 „	+	0·012	+	0·55	+	0·05	=	+ + + +
(3)	1 „	+	0·012	+	0·5	+	0·1	=	+ + + +
(4)	1 „	+	0·012	+	0·45	+	0·15	=	+ + + +
(5)	1 „	+	0·012	+	0·4	+	0·2	=	+ +
(6)	1 „	+	0·012	+	0·35	+	0·25	=	+
(7)	1 „	+	0·012	+	0	+	0·6	=	0

The mixtures were kept at 37° C. for 1½ hour.

In the serum obtained before (as well as after) injection the M.H.D. of complement could not be determined exactly—complete hæmolysis not having occurred in the above set of tubes, and there not being enough of serum left in either case to do a further set. But 0·6 c.c. of the 1 in 3 dilution of complement with 0·012 c.c. of the diluted heated immune serum produced almost complete hæmolysis in 1 c.c. of a 1 per cent. suspension of ox blood corpuscles, while 0·35 c.c. of the diluted complement produced a trace in a similar mixture. In the serum obtained after injection the series of tubes prepared in exactly the same way in every detail show exactly the same result. If we take 0·7 c.c. of the diluted complement as the M.H.D. for 1 c.c. 1 per cent. suspension of ox blood corpuscles, then the 2 c.c. of ox blood corpuscles injected ought to have used up 46 c.c. of complement; while, taking the blood volume as 4·85 per cent. of the body weight and the complement = 50 per cent. of the blood, the total complement in the rabbit must in reality have been about 48 c.c.

Experiment 2.

Rabbit, female; weight, 2220 grms.; ox blood corpuscles washed six times; M.H.D. of heated immune body to sensitise them was 0·01 c.c. of a 1 in 6 dilution for 2 c.c. of a 1 per cent. suspension of ox blood corpuscles. The rabbit was bled from the ear vein to the extent of 15 c.c.; and then immediately a mixture of 3 c.c. corpuscles + 0·24 c.c. heated immune serum + 2 c.c. saline, which had been kept at 37 C. for one and a half hour was injected into the ear vein. This animal again showed no symptoms of distress after injection, and one and a half hour after injection 15 c.c. blood were again drawn from the ear vein. The sera were allowed to separate as before, and the M.H.D. determined as before. The serum after injection was again darkly hæmoglobin tinged.

Determination of Complement.

Complement was undiluted; heated immune serum was diluted 1 in 6 with 0·85 per cent. saline. M.H.D. for 2 c.c. 1 per cent. suspension of ox blood corpuscles = 0·01 c.c.; 1 per cent. suspension of ox blood corpuscles was used.

Before Injection.

	1 per cent. Suspension O.B.C.	H.I.S.	Compt.	0·85 per cent. Saline.	
(1)	2 c.c.	+ 0·01	+ 0·5	+ 0	= C.[1]
(2)	2 „	+ 0·01	+ 0·45	+ 0·05	= C.
(3)	2 „	+ 0·01	+ 0·4	+ 0·1	= C.
(4)	2 „	+ 0·01	+ 0·375	+ 0·125	= C.
(5)	2 „	+ 0·01	+ 0·35	+ 0·15	= C.
(6)	2 „	+ 0·01	+ 0·325	+ 0·175	= C.
(7)	2 „	+ 0·01	+ 0·3	+ 0·2	= C.
(8)	2 „	+ 0·01	+ 0·25	+ 0·25	= + + + +
(9)	2 „	+ 0·01	+ 0·2	+ 0·3	= + + + +
(10)	2 „	+ 0·01	+ 0	+ 0·5	= 0

[1] C. = 1½ hour at 37° C.

After Injection.

(1)	2 c.c.	+ 0·01	+ 0·5	+ 0	= C.
(2)	2 „	+ 0·01	+ 0·45	+ 0·05	= C.
(3)	2 „	+ 0·01	+ 0·4	+ 0·1	= C.
(4)	2 „	+ 0·01	+ 0·375	+ 0·125	= C.
(5)	2 „	+ 0·01	+ 0·35	+ 0·15	= C.
(6)	2 „	+ 0·01	+ 0·325	+ 0·175	= C.
(7)	2 „	+ 0·01	+ 0·3	+ 0·2	= C.
(8)	2 „	+ 0·01	+ 0·25	+ 0·25	= + + + +
(9)	2 „	+ 0·01	+ 0·2	+ 0·3	= + + + +
(10)	2 „	+ 0·01	+ 0	+ 0·5	= 0

1½ hour at 37° C.

The M.H.D. of complement before injection for 2 c.c. 1 per cent. ox blood corpuscle suspension sensitised with 0·01 c.c. of a dilution of 1 in 6 heated immune serum is therefore 0·3 c.c., and the M.H.D. of complement after injection under the same circumstances is exactly the same; and the results of the two series of determinations is exactly the same in every detail. Here, if 0·3 c.c. complement is taken as the M.H.D. of complement for 2 c.c. of a 1 per cent. suspension of ox blood corpuscles, then the 3 c.c. ox blood corpuscles injection ought to have used up 45 c.c. complement, and, calculated as before, the animal being a doe, the total amount of complement present in its body amounted to 58 c.c.

Series II.

To determine the change in complement content of the serum of a fresh rabbit, after injection intravenously of ox blood corpuscles *sensitised to saturation*, the possibility of which was originally pointed out by Ehrlich and Morgenroth (1900). An example of the method of obtaining saturation of corpuscles may be here given.

The M.H.D. of heated immune serum for 2 c.c. 1 per cent. suspension of ox blood corpuscles is 0·05 c.c. of a 1 in 6 dilution when the mixture is fully complemented. One per cent. ox blood corpuscle suspension used. Complement added in excess undiluted.

	Ox Blood Corpuscle Suspension.		H.I.S.			Saline.	
(1)	2 c.c.	+	0·05 c.c.	(1 M.H.D.)	+	0·25 c.c.	Incubated one and a half hour at 37°C.; then centrifugalised and supernatant fluid added to deposit from 2 c.c. 1 per cent. suspension of fresh ox blood corpuscle.
(2)	2 „	+	0·1 „	(2 „)	+	0·20 „	
(3)	2 „	+	0·15 „	(3 „)	+	0·15 „	
(4)	2 „	+	0·20 „	(4 „)	+	0·1 „	
(5)	2 „	+	0·25 „	(5 „)	+	0·05 „	
(6)	2 „	+	0·30 „	(6 „)	+	0 „	

	Supernatant Fluid + Fresh Ox Blood Corpuscles.		Complement.		
(1)	2 c.c.	+	0·5 c.c.	=	0
(2)	2 „	+	0·5 „	=	0
(3)	2 „	+	0·5 „	=	+
(4)	2 „	+	0·5 „	=	+ + +
(5)	2 „	+	0·5 „	=	C.
(6)	2 „	+	0·5 „	=	C.

This mixture was kept in the incubator at 37° C. for one and a half hour. It will be seen that the corpuscles took up at least 2 M.H.D. of heated immune serum.

Experiment 1.

Rabbit, doe; weight, 1460 grms.; ox blood corpuscles washed six times. M.H.D. of the heated immune serum used was 0·05 of a 1 in 6 dilution for 2 c.c. 1 per cent. suspension of ox blood corpuscles. For saturation the corpuscles were given 3 M.H.D.

The rabbit was bled to the extent of 15 c.c. It was then injected intravenously with a mixture of 3·48 c.c. heated immune serum (= 3 M.H.D.) + 2·8 c.c. corpuscles. The mixture had been kept in the incubator for one and a half hour and the whole mixture (heated immune serum and corpuscles) was injected. After injection the rabbit was quite lively, and showed no symptoms of distress. A sample of blood was removed again in one and a half hour. The sera were allowed to separate as before. The serum obtained after injection was deeply hæmoglobin tinted.

To determine the M.H.D. of complement. Complement was undiluted; heated immune serum was diluted 1 in 6; M.H.D. = 0·05 c.c., for 2 c.c. 1 per cent. suspension of ox blood corpuscles. One per cent. suspension of ox blood corpuscles used.

Before Injection.

	1 per cent. Suspension O.B.C.		H.I.S.		Compt.		Saline.		
(1)	2 c.c.	+	0·05	+	0·5	+	0	=	+ + +
(2)	2 „	+	0·05	+	0·4	+	0·1	=	+ + +
(3)	2 „	+	0·05	+	0·35	+	0·15	=	+ +
(4)	2 „	+	0·05	+	0·3	+	0·2	=	+ +
(5)	2 „	+	0·05	+	0·25	+	0·25	=	+
(6)	2 „	+	0·05	+	0·2	+	0·3	=	0
(7)	2 „	+	0·05	+	0·15	+	0·35	=	0
(8)	2 „	+	0·05	+	0·1	+	0·4	=	0
(9)	2 „	+	0·05	+	0	+	0·5	=	0

1½ hour at 37° C.

After Injection.

	1 per cent. Suspension O.B.C.		H.I.S.		Compt.		Saline.		
(1)	2 c.c.	+	0·05	+	0·5	+	0	=	+ + +
(2)	2 „	+	0·05	+	0·4	+	0·1	=	+ + +
(3)	2 „	+	0·05	+	0·35	+	0·15	=	+ +
(4)	2 „	+	0·05	+	0·3	+	0·2	=	+ +
(5)	2 „	+	0·05	+	0·25	+	0·25	=	+
(6)	2 „	+	0·05	+	0·2	+	0·3	=	0
(7)	2 „	+	0·05	+	0·15	+	0·35	=	0
(8)	2 „	+	0·05	+	0·1	+	0·4	=	0
(9)	2 „	+	0·05	+	0	+	0·5	=	0

1¼ hour at 37° C.

Thus in the serum obtained before injection the M.H.D. of complement is not arrived at in the extent of the experiment, but hæmolysis is seen to start with 0·25 c.c. complement. In the serum obtained after injection hæmolysis starts also with 0·25 c.c. complement, and the two series of results are exactly the same in every respect. Here, if 0·6 c.c. complement be taken as the M.H.D. of complement for 2 c.c. 1 per cent. ox blood corpuscle suspension, then the 2·8 c.c. of ox corpuscles injected ought to have used up 84 c.c. of the complement, and, calculated as before, the total amount of complement present in the animal's body only amounted to 38·6 c.c.

Experiment 2.

Rabbit, buck, weight, 2220 grms. Ox blood corpuscles were washed six times. M.H.D. of the heated immune serum was 0·01 c.c. of a 1 in 6 dilution for 1 c.c. of a 1 per cent. suspension. For saturation the corpuscles were given 6 M.H.D. of the heated immune serum.

The rabbit was bled to the extent of 15 c.c. It was then injected intravenously with a mixture of 4 c.c. heated immune serum (6 M.H.D.) + 4 c.c. ox blood corpuscles which had been incubated for one and a half hour at 37° C. The rabbit showed great dyspnœa after injection, and was lying down in great distress. It recovered a little, and was living at the end of one and a half hour. At this time blood was obtained from the internal jugular. Post-mortem, there were a few hæmorrhages in the lung, and a few petechiæ subperitoneally. The urine contained abundant hæmoglobin. The corpuscular suspension used for injection contained minute flocculi visible to the naked eye, and this may explain the symptoms and post-mortem appearances. There was no air in the heart. The sera were allowed to separate as before. The serum after injection was deeply hæmoglobin tinted. The M.H.D. of the complement was then determined. In this determination complement was used undiluted; heated immune serum was diluted 1 in 6, of which M.H.D. = 0·01 c.c. for 1 c.c. 1 per cent. suspension of ox blood corpuscles; 1 per cent. suspension of ox blood corpuscles used.

Complement before Injection.

	1 per cent. Suspension O.B.C.		H.I.S.		Compt.		Saline.		
(1)	1 c.c.	+	0·01	+	0·4	+	0	=	C.
(2)	1 ,,	+	0·01	+	0·35	+	0·05	=	C.
(3)	1 ,,	+	0·01	+	0·03	+	0·01	=	C.
(4)	1 ,,	+	0·01	+	0·25	+	0·15	=	C.
(5)	1 ,,	+	0·01	+	0·02	+	0·02	=	C.
(6)	1 ,,	+	0·01	+	0·15	+	0·25	=	C.
(7)	1 ,,	+	0·01	+	0·1	+	0·3	=	C.
(8)	1 ,,	+	0·01	+	0·05	+	0·35	=	+ + + +
(9)	1 ,,	+	0·01	+	0·025	+	0·375	=	+
(10)	1 ,,	+	0·01	+	0	+	0·4	=	0

1½ hour at 37° C.

After Injection.

(1)	1 ,,	+	0·01	+	0·4	+	0	=	C.
(2)	1 ,,	+	0·01	+	0·35	+	0·05	=	C.
(3)	1 ,,	+	0·01	+	0·3	+	0·1	=	C.
(4)	1 ,,	+	0·01	+	0·25	+	0·15	=	C.
(5)	1 ,,	+	0·01	+	0·2	+	0·2	=	C.
(6)	1 ,,	+	0·01	+	0·15	+	0·25	=	C.
(7)	1 ,,	+	0·01	+	0·1	+	0·3	=	C.
(8)	1 ,,	+	0·01	+	0·05	+	0·35	=	+ + + +
(9)	1 ,,	+	0·01	+	0·025	+	0·375	=	+
(10)	1 ,,	+	0·01	+	0	+	0·4	=	0

1½ hour at 37° C.

Thus in the serum obtained before injection the M.H.D. of complement for 1 c.c. 1 per cent. suspension of ox blood corpuscles sensitised with 0·01 c.c. of the diluted treated immune serum is 0·1 c.c. In the serum obtained after injection the M.H.D. of the complement is the same. Here, if we take 0·1 c.c. complement as the M.H.D. of complement for 1 c.c. of 1 per cent. suspension of ox blood corpuscles, then the 4 c.c. corpuscles injected ought to have used up 40 c.c. complement, and the amount of complement present in the blood of the rabbit calculated as before is 53·8 c.c.

Series III.

To determine the change, if any, in complement content of the serum of a fresh rabbit after injection intravenously of *unsensitised* ox blood corpuscles.

Experiment 1.

Rabbit, buck; weight, 2320. Its serum contained no lysin for ox blood corpuscles. The rabbit was bled to the extent of 15 c.c. It was then injected intravenously with 4 c.c. ox blood corpuscles + 2 c.c. saline. It showed no symptoms of distress, and one and a half hour after injection it was bled again to the extent of 15 c.c. The sera were separated as before. The serum obtained after injection had only the slightest hæmoglobin tint.

Determination of the M.H.D. of Complement.

Complement was used undiluted; heated immune serum was diluted 1 in 6 M.H.D. for 2 c.c. One per cent. suspension of ox blood corpuscles = 0·06 of a suspension 1 in 6 dilution. One per cent. suspension of ox blood corpuscles used.

Before Injection.

	1 per cent. Suspensions O.B.C.	H.I.S.	Compt.	Saline.	
(1)	2 c.c.	+ 0·06	+ 0·5	+ 0	= C.
(2)	2 ,,	+ 0·06	+ 0·45	+ 0·05	= C.
(3)	2 ,,	+ 0·06	+ 0·4	+ 0·1	= C.
(4)	2 ,,	+ 0·06	+ 0·35	+ 0·15	= C.
(5)	2 ,,	+ 0·06	+ 0·3	+ 0·2	= C.
(6)	2 ,,	+ 0·06	+ 0·25	+ 0·25	= C.
(7)	2 ,,	+ 0·06	+ 0·2	+ 0·3	= C.
(8)	2 ,,	+ 0·06	+ 0·15	+ 0·35	= ++++
(9)	2 ,,	+ 0·06	+ 0·1	+ 0·4	= +++
(10)	2 ,,	+ 0·06	+ 0	+ 0·5	= 0

1½ hour at 37° C.

After Injection.

(1)	2 c.c.	+ 0·06	+ 0·5	+ 0	= C.
(2)	2 ,,	+ 0·06	+ 0·45	+ 0·05	= C.
(3)	2 ,,	+ 0·06	+ 0·4	+ 0·1	= C.
(4)	2 ,,	+ 0·06	+ 0·35	+ 0·15	= C.
(5)	2 ,,	+ 0·06	+ 0·3	+ 0·2	= C.
(6)	2 ,,	+ 0·06	+ 0·25	+ 0·25	= C.
(7)	2 ,,	+ 0·06	+ 0·2	+ 0·3	= C.
(8)	2 ,,	+ 0·06	+ 0·15	+ 0·35	= ++++
(9)	2 ,,	+ 0·06	+ 0·1	+ 0·4	= +++
(10)	2 ,,	+ 0·06	+ 0	+ 0·5	= 0

1½ hour at 37° C.

Thus in the serum obtained before injection the M.H.D. of complement for 2 c.c. 1 per cent. suspension of ox blood corpuscles is 0·2 c.c., and in the serum obtained after injection the M.H.D. is the same. Here, if we take 0·2 c.c. complement as the M.H.D. of complement for 2 c.c. 1 per cent. suspension of ox blood corpuscles, then the 4 c.c. ox blood corpuscles injected might have used up 40 c.c. complement, and the amount of complement present in the blood of the rabbit calculated as before amounts to 56 c.c.

Experiment 2.

Rabbit, buck; weight, 1900 grms. Serum contains normally no lysin for ox blood corpuscles. The rabbit was bled to the extent of 15 c.c. It was then injected intravenously with 3 c.c. ox blood corpuscles + 2 c.c. saline. It showed no signs of distress. One and a half hour after injection it was bled again to the extent of 15 c.c. The sera were then separated as before. The serum obtained after injection had only the slightest hæmoglobin tint.

Determination of the M.H.D. of Complement.

Complement undiluted. Heated immune serum was diluted 1 in 6 ; of which M.H.D. for 2 c.c. 1 per cent. suspension of ox blood corpuscles = 0·06 c.c. One per cent. suspension of ox blood corpuscles used.

Before Injection.

	1 per cent. Suspension O.B.C.	H.I.S.	Compt.	Saline.	
(1)	2 c.c.	+ 0·06	+ 0·5	+ 0	= C.
(2)	2 „	+ 0·06	+ 0·45	+ 0·05	= C.
(3)	2 „	+ 0·06	+ 0·4	+ 0·1	= C.
(4)	2 „	+ 0·06	+ 0·35	+ 0·15	= C.
(5)	2 „	+ 0·06	+ 0·3	+ 0·2	= C.
(6)	2 „	+ 0·06	+ 0·25	+ 0·25	= C.
(7)	2 „	+ 0·06	+ 0·2	+ 0·3	= + +
(8)	2 „	+ 0·06	+ 0·15	+ 0·35	= + ?[1]
(9)	2 „	+ 0·06	+ 0·1	+ 0·4	= + ?
(10)	2 „	+ 0.06	+ 0	+ 0·5	= 0

1½ hour at 37° C.

After Injection.

(1)	2 c.c.	+ 0·06	+ 0·5	+ 0	= C.
(2)	2 „	+ 0·06	+ 0·45	+ 0·05	= C.
(3)	2 „	+ 0·06	+ 0·4	+ 0·1	= C.
(4)	2 „	+ 0·06	+ 0·35	+ 0·15	= C.
(5)	2 „	+ 0·06	+ 0·3	+ 0·2	= C.
(6)	2 „	+ 0·06	+ 0·25	+ 0·25	= C.
(7)	2 „	+ 0·06	+ 0·2	+ 0·3	= + +
(8)	2 „	+ 0·06	+ 0·15	+ 0·35	= + ?[1]
(9)	2 „	+ 0·06	+ 0·1	+ 0·4	= + ?
(10)	2 „	+ 0·06	+ 0	+ 0·5	= 0

1½ hour at 37° C.

Thus in the serum obtained before injection the M.H.D. of complement for 2 c.c. 1 per cent. suspension of ox blood corpuscles is 0·25 c.c. In the serum obtained after injection the M.H.D. is the same. Here, if we take 0·25 c.c. as the M.H.D. of complement for 2 c.c. 1 per cent. suspension of ox blood corpuscles, then the 3 c.c. corpuscles injected might have used up 37·5 c.c. complement ; and the amount of complement present in the blood of the rabbit calculated as before amounts to 46 c.c.

Series IV.

To determine the change in complement content of the serum of an *immune* rabbit after injection intravenously of *completely saturated* ox blood corpuscles

In determining the M.H.D. of the complement in the immune rabbit, after the injection of corpuscles the ox blood corpuscles

[1] Trace of hæmolysis doubtful.

were saturated with heated immune body (as compared with their being merely sensitised, as was done in the fresh rabbits). This was in order to make them independent of the immune body in the serum whose complement was to be determined. The other steps were the same as for the determination of M.H.D. of complement in fresh rabbits.

Experiment 1.

Rabbit, buck; weight, 2150 grms. This rabbit had been immunised for several weeks, and its serum contained immune body to such an amount that 0·005 c.c. when fully complemented hæmolysed completely 2 c.c. of a 1 per cent. suspension of ox blood corpuscles. The heated immune serum used for saturating the corpuscles had a M.H.D. for 2 c.c. 1 per cent. suspension of ox blood corpuscles of 0·01 c.c.; for saturation the corpuscles took up 3 M.H.D. The rabbit was bled to the extent of 15 c.c. It was then injected intravenously with a mixture of 4·5 c.c. heated immune serum (= 3 M.H.D.) + 3 c.c. ox blood corpuscles. The mixture had been kept in the incubator for one and a half hour at 37° C. The rabbit was very ill after injection and was dying when, after one and a half hour, blood was obtained from its jugular. The serum was allowed to separate as before. The serum after injection was darkly hæmoglobin stained.

Determination of the M.H.D. of Complement.

Complement was undiluted. Heated immune serum was diluted 1 in 6. M.H.D. = 0·06 for 2 c.c. 1 per cent. suspension of ox blood corpuscles. Three M.H.D. used to each tube to saturate the corpuscles and make them independent of the immune body in the serum whose complement was to be determined.

Before Injection.

	1 per cent. Suspension O.B.C.		H.I.S.		Compt.		Saline.		
(1)	2 c.c.	+	0·18	+	0·5	+	0	=	C.
(2)	2 ,,	+	0·18	+	0·45	+	0·05	=	C.
(3)	2 ,,	+	0·18	+	0·4	+	0·1	=	C.
(4)	2 ,,	+	0·18	+	0·375	+	0·125	=	C.
(5)	2 ,,	+	0·18	+	0·35	+	0·15	=	C.
(6)	2 ,,	+	0·18	+	0·32	+	0·175	=	C.
(7)	2 ,,	+	0·18	+	0·3	+	0·2	=	C.
(8)	2 ,,	+	0·18	+	0·25	+	0·25	=	C.
(9)	2 ,,	+	0·18	+	0·2	+	0·3	=	C.
(10)	2 ,,	+	0·18	+	0	+	0·5	=	0
(11)	2 ,,	+	0·18	+	0·15	+	0	=	C.
(12)	2 ,,	+	0·18	+	0·1	+	0·05	=	+ + + +
(13)	2 ,,	+	0·18	+	0·09	+	0·06	=	+ + + +
(14)	2 ,,	+	0·18	+	0·08	+	0·07	=	+ + + +
(15)	2 ,,	+	0·18	+	0·07	+	0·08	=	+ + + +
(16)	2 ,,	+	0·18	+	0·06	+	0·09	=	+ + + +
(17)	2 ,,	+	0·18	+	0·05	+	0·1	=	+ + +
(18)	2 ,,	+	0·18	+	0·04	+	0·11	=	+ + +
(19)	2 ,,	+	0·18	+	0·03	+	0·12	=	+ ?
(20)	2 ,,	+	0·18	+	0	+	0·15	=	0

1½ hour at 37° C.

After Injection.

	1 per cent. Suspension O.B.C.	H.I.S.	Compt.	Saline.		
(1)	2 c.c.	+ 0·18	+ 0·6	+ 0	=	+ + +
(2)	2 „	+ 0·18	+ 0·5	+ 0·1	=	+ + +
(3)	2 „	+ 0·18	+ 0·4	+ 0·2	=	+ +
(4)	2 „	+ 0·18	+ 0·3	+ 0·3	=	+ +
(5)	2 „	+ 0·18	+ 0·2	+ 0·4	=	+
(6)	2 „	+ 0·18	+ 0·15	+ 0	=	+
(7)	2 „	+ 0·18	+ 0·1	+ 0·05	=	?
(8)	2 „	+ 0·18	+ 0·09	+ 0·06	=	?
(9)	2 „	+ 0·18	+ 0·08	+ 0·07	=	?
(10)	2 „	+ 0·18	+ 0·07	+ 0·08	=	0
(11)	2 „	+ 0·18	+ 0·06	+ 0·09	=	0
(12)	2 „	+ 0·18	+ 0·05	+ 0·01	=	0
(13)	2 „	+ 0·18	+ 0·04	+ 0·11	=	0
(14)	2 „	+ 0·18	+ 0·03	+ 0·12	=	0
(15)	2 „	+ 0·18	+ 0	+ 0·15	=	0

1½ hour at 37° C.

Thus in the serum obtained before injection the M.H.D. of complement for 2 c.c. 1 per cent. suspension of ox blood corpuscles is 0·15 c.c.; while in the serum after injection 0·6 c.c. of complement does not produce nearly complete hæmolysis in a like quantity of the suspension. Here, if we take 0·15 c.c. as the M.H.D. of complement for 2 c.c. 1 per cent. suspension of ox blood corpuscles, then the 3 c.c. corpuscles ought to have used up 22·5 c.c. of the complement; and the amount of complement present in the blood of the rabbit calculated as before amounts to 52 c.c.

Experiment 2.

Rabbit, buck; weight, 2250 grms. This rabbit's serum contained immune body to such an extent that 0·01 c.c. serum when fully complemented completely hæmolysed 2 c.c. 1 per cent. suspension of ox blood corpuscles. For saturation the corpuscles took up 3 M.H.D. of heated immune serum. The rabbit was bled to the extent of 15 c.c. Then a mixture of 4 c.c. ox corpuscles + 6 c.c. heated immune serum (3 M.H.D.) was injected intravenously. The mixture had been kept one and a half hour in the incubator. The rabbit had great dyspnœa and distress, and died a few minutes before the expiration of the one and a half hour it was to be allowed to live. Blood was obtained from the heart. Post-mortem it showed a large infarction of the small intestine. The serum was allowed to separate as before. The serum obtained after injection was darkly hæmoglobin tinted.

Determination of the M.H.D. of Complement.

Complement was undiluted. Heated immune serum was diluted 1 in 6; 3 M.H.D. were used to saturate the corpuscles to make them independent of the immune body in the serum whose complement was to be determined. M.H.D. of immune body for 2 c.c. 1 per cent. suspension of ox blood corpuscles = 0·06 of a 1 in 6 dilution.

Complement before Injection.

	1 per cent. Suspension O.B.C.	H.I.S.	Compt.	Saline.	
(1)	2 c.c.	+ 0·18	+ 0·5	+ 0	= C.
(2)	2 „	+ 0·18	+ 0·4	+ 0·1	= C.
(3)	2 „	+ 0·18	+ 0·3	+ 0·2	= C.
(4)	2 „	+ 0·18	+ 0·2	+ 0·3	= C.
(5)	2 „	+ 0·18	+ 0·1	+ 0·4	= C.
(6)	2 „	+ 0·18	+ 0·08	+ 0·42	= + + + +
(7)	2 „	+ 0·18	+ 0·06	+ 0·44	= + +
(8)	2 „	+ 0·18	+ 0·04	+ 0·46	= +
(9)	2 „	+ 0·18	+ 0·01	+ 0·49	= 0
(10)	2 „	+ 0·18	+ 0	+ 0·5	= 0

1½ hour at 37° C.

Complement after Injection.

(1)	2 c.c.	+ 0·18	+ 0·5	+ 0	= + + +
(2)	2 „	+ 0·18	+ 0·4	+ 0·1	= + +
(3)	2 „	+ 0·18	+ 0·3	+ 0·2	= + +
(4)	2 „	+ 0·18	+ 0·2	+ 0·3	= +
(5)	2 „	+ 0·18	+ 0·1	+ 0·4	= 0
(6)	2 „	+ 0·18	+ 0·08	+ 0·42	= 0
(7)	2 „	+ 0·18	+ 0·06	+ 0·44	= 0
(8)	2 „	+ 0·18	+ 0·04	+ 0·46	= 0
(9)	2 „	+ 0·18	+ 0·01	+ 0·49	= 0
(10)	2 „	+ 0·18	+ 0	+ 0·5	= 0

1½ hour at 37° C.

Thus in the serum obtained before injection the M.H.D. of complement for 2 c.c. 1 per cent. suspension of ox blood corpuscles is 0·1 c.c., while in the serum obtained after injection 0·5 c.c. complement produced practically only about half hæmolysis. Here, if we take 0·1 c.c. as the M.H.D. of complement for 2 c.c. 1 per cent. suspension of ox blood corpuscles, then the 4 c.c. ox corpuscles injected ought to have used up 20 c.c. of complement; and the amount of complement present in the blood of the rabbit, calculated as before, amounts to 54 c.c.

Series V.

To determine the change in complement content of an *immune* rabbit after intravenous injection of fresh unsensitised ox blood corpuscles.

Experiment 1.

Rabbit, buck; weight, 2130 grms. This rabbit had been immunised for about six months with ox blood corpuscles. The M.H.D. of its immune body for 2 c.c. 1 per cent. suspension of ox blood corpuscles fully complemented was 0·0025 c.c.

The rabbit was bled to the extent of 15 c.c. Then 4·5 c.c. ox blood corpuscles + 2 c.c. saline, warmed in the incubator, were injected into the ear vein. The rabbit died in five minutes. Post-mortem there was no air found in the heart and no lesion was seen in any of the organs. The liver, lung,

spleen, and kidney were examined microscopically and no signs of infarction seen. Blood was obtained from the heart. The sera were separated as before. The serum after injection was deeply hæmoglobin tinted.

Determination of the M.H.D. of Complement.

Complement was undiluted; heated immune serum was diluted 1 in 10, and 3 M.H.D. were used to saturate corpuscles to make them independent of the immune body introduced with the complement. M.H.D. of immune body = 0·025 c.c. of 1 in 10 dilution for 1 c.c. 1 per cent. supension. One per cent. suspension of ox blood corpuscles used.

Complement before Injection.

	1 per cent. Suspension O.B.C.		H.I.S.		Compt.		Saline.		
(1)	1 c.c.	+	0·075	+	0·5	+	0	=	C.
(2)	1 „	+	0·075	+	0·4	+	0·1	=	C.
(3)	1 „	+	0·075	+	0·3	+	0·2	=	C.
(4)	1 „	+	0·075	+	0·2	+	0·3	=	C.
(5)	1 „	+	0·075	+	0·1	+	0·4	=	C.
(6)	1 „	+	0·075	+	0·08	+	0·42	=	C.
(7)	1 „	+	0·075	+	0·06	+	0·44	=	C.
(8)	1 „	+	0·075	+	0·04	+	0·46	=	C.
(9)	1 „	+	0·075	+	0·01	+	0·49	=	+
(10)	1 „	+	0·075	+	0	+	0·5	=	0

1½ hour at 37° C.

Complement after Injection.

(1)	1 c.c.	+	0·075	+	0·5	+	0	=	+
(2)	1 „	+	0·075	+	0·4	+	0·1	=	+
(3)	1 „	+	0·075	+	0·3	+	0·2	=	+
(4)	1 „	+	0·075	+	0·2	+	0·3	=	+
(5)	1 „	+	0·075	+	0·1	+	0·4	=	+
(6)	1 „	+	0·075	+	0·08	+	0·42	=	+
(7)	1 „	+	0·075	+	0·06	+	0·44	=	+
(8)	1 „	+	0·075	+	0·04	+	0·46	=	0
(9)	1 „	+	0·075	+	0·01	+	0·49	=	0
(10)	1 „	+	0·075	+	0	+	0·5	=	0

1½ hour at 37° C.

Thus in the serum obtained before injection the M.H.D. of complement was 0·04 c.c. for 1 c.c. 1 per cent. suspension, while in the serum after injection 0·5 c.c. complement produced only a trace of hæmolysis. Here, if we take 0·04 c.c. as the M.H.D. of complement for 1 c.c. 1 per cent. suspension of ox blood corpuscles, then the 4·5 c.c. corpuscles injected ought to have used up 18 c.c. complement: and the amount of the complement in the blood of the rabbit, calculated as before, amounts to 51 c.c.

Experiment 2.

Rabbit, buck; weight, 2370 grms. This rabbit's serum contains immune body to such an extent that 0·07 of a 1 in 10 dilution when fully complemented hæmolysed 1 c.c. of a 1 per cent. suspension of ox blood corpuscles.

The rabbit was bled to the extent of 15 c.c., it was then injected with 4·5 c.c. ox blood corpuscles + 2 c.c. saline which had been warmed in the incubator. It became very ill two minutes after injection with great dyspnœa, got gradually better, and when killed was almost recovered. Post-mortem, no hæmorrhages were seen anywhere. Hæmoglobin was present in the urine. The sera were allowed to separate as before. The serum obtained after injection was darkly hæmoglobin tinted.

Determination of the M.H.D. of Complement.

Complement was undiluted. Immune body undiluted, and about four times M.H.D. added to corpuscles to saturate them; *i.e.* 0·03 c.c. of the undiluted serum added to 1 c.c. 1 per cent. suspension of ox blood corpuscles. One per cent. suspension of ox blood corpuscles used.

Complement before Injection.

	1 per cent. Suspension O.B.C.		H.I.S.		Compt.		Saline.		
(1)	1 c.c.	+	0·03	+	0·5	+	0	=	C.
(2)	1 „	+	0·03	+	0·4	+	0·1	=	C.
(3)	1 „	+	0·03	+	0·3	+	0·2	=	C.
(4)	1 „	+	0·03	+	0·2	+	0·3	=	C.
(5)	1 „	+	0·03	+	0·1	+	0·4	=	C.
(6)	1 „	+	0 03	+	0·08	+	0·42	=	+ + + +
(7)	1 „	+	0·03	+	0·06	+	0·44	=	+ + +
(8)	1 „	+	0·03	+	0·04	+	0·46	=	+
(9)	1 „	+	0·03	+	0·01	+	0·49	=	0
(10)	1 „	+	0·03	+	0	+	0·5	=	0

1½ hour at 37° C.

Complement after Injection.

(1)	1 c.c.	+	0·03	+	0·5	+	0·0	=	C
(2)	1 „	+	0·03	+	0·4	+	0·1	=	C.
(3)	1 „	+	0·03	+	0·3	+	0·2	=	C.
(4)	1 „	+	0·03	+	0·2	+	0·3	=	C.
(5)	1 „	+	0·03	+	0·1	+	0·4	=	+
(6)	1 „	+	0·03	+	0·08	+	0·42	=	0
(7)	1 „	+	0·03	+	0·06	+	0·44	=	0
(8)	1 „	+	0·03	+	0·04	+	0·46	=	0
(9)	1 „	+	0·03	+	0·01	+	0·49	=	0
(10)	1 „	+	0·03	+	0	+	0·5	=	0

1½ hour at 37° C.

Thus in the serum obtained before injection the M.H.D. of complement was 0·1 c.c. for 1 c.c. 1 per cent. suspension of ox blood corpuscles, and there was a trace of hæmolysis with 0·04 c.c.; while in the serum after injection the hæmolysis was nearly complete with 0·2 c.c. complement for a like quantity of ox blood corpuscles, while with 0·1 c.c. there was only a trace, and with 0·08, 0·06, and 0·04 there was no hæmolysis. Here, if we take 0·1 c.c. as the M.H.D. for 1 c.c. 1 per cent. suspension of ox blood corpuscles, then the 4 c.c. ox blood corpuscles injected ought to have used up 45 c.c. complement,

and the amount of complement present in the blood of the rabbit calculated as before amounts to 57 c.c.

It will be well here (1) to bring forward evidence that the change in the complementing action of the serum in Series IV. and V. is really due to a diminution in complement, and not due to some action produced *in vivo* by the large excess of immune body in the complementing serum; (2) to show by an experiment how the minimal hæmolytic dose of complement is much lowered by adding *in vitro* an excess of immune body.

As regards (1)—that the change in the complementing power of the serum is due to a diminution in complement—this cannot be proved directly, but indirect proof can be got by determining the complement content of the blood of a fresh rabbit before and after injection of saturated corpuscles, and by using in this determination a large excess of heated immune body. It will be seen that the complement content of the rabbit's blood is exactly the same before and after injection—a condition which was also found in Series II., where a fresh rabbit was similarly injected, and where only one M.H.D. of heated immune body was used in the determination of the complement before and after injection.

Experiment.

Rabbit, buck; weight, 1850 grms. Bled 10 c.c. at 10.30; then injected at 10.40 intravenously with 3 c.c. ox blood corpuscles + 1·35 c.c. heated immune serum, the mixture having been in incubator at 37° C. for one and a half hour. (M.H.D. of heated immune serum for 1 c.c. 1 per cent. suspension of ox blood corpuscles = 0·0015 heated immune serum; here the corpuscles were saturated with 3 M.H.D.) Blood obtained after injection at 12.10; serum separated as before; serum after injection deeply hæmoglobin tinted.

Determination of the Complement.

Complement was undiluted. Heated immune serum (whose M.H.D. for 1 c.c. 1 per cent. suspension of ox blood corpuscles = 0·0015 c.c.) used in a dilution of 1 in 20 and in dose of 3 M.H.D. for sensitising to saturation the corpuscles, and used full strength in doses of 0·3 c.c. for the purpose of adding great excess of immune body to the various test mixtures. One per cent. ox blood corpuscles used.

Before Injection.

	1 per cent. O.B.C.		H.I.S. 1 in 20.		Compt.		H.I.S. undil.		Saline.	
(1)	1 c.c.	+	0·09 c.c.	+	0·5 c.c.	+	0·3 c.c.	+	0	= C.
(2)	1 „	+	0·09 „	+	0·4 „	+	0·3 „	+	0·1	= C.
(3)	1 „	+	0·09 „	+	0·3 „	+	0·3 „	+	0·2	= C.
(4)	1 „	+	0·09 „	+	0·2 „	+	0·3 „	+	0·3	= C.
(5)	1 „	+	0·09 „	+	0·1 „	+	0·3 „	+	0·4	= C.
(6)	1 „	+	0·09 „	+	0·08 „	+	0·3 „	+	0·42	= C.
(7)	1 „	+	0·09 „	+	0·06 „	+	0·3 „	+	0·44	= C.
(8)	1 „	+	0·09 „	+	0·04 „	+	0·3 „	+	0·46	= C.
(9)	1 „	+	0·09 „	+	0·02 „	+	0·3 „	+	0·48	= + + +
(10)	1 „	+	0·09 „	+	0 „	+	0·3 „	+	0·5	= 0

1½ hour at 37° C.

After Injection.

	1 per cent. O.B.C.		H.I.S. 1 in 20.		Compt.		H.I.S.		Saline.		
(1)	1 c.c.	+	0·09 c.c.	+	0·5 c.c.	+	0·3 c.c.	+	0	=	C.
(2)	1 „	+	0·09 „	+	0·4 „	+	0·3 „	+	0·1	=	C.
(3)	1 „	+	0·09 „	+	0·3 „	+	0·3 „	+	0·2	=	C.
(4)	1 „	+	0·09 „	+	0·2 „	+	0·3 „	+	0·3	=	C.
(5)	1 „	+	0·09 „	+	0·1 „	+	0·3 „	+	0·4	=	C.
(6)	1 „	+	0·09 „	+	0·08 „	+	0·3 „	+	0·42	=	C.
(7)	1 „	+	0·09 „	+	0·06 „	+	0·3 „	+	0·44	=	C.
(8)	1 „	+	0·09 „	+	0·04 „	+	0·3 „	+	0·46	=	C.
(9)	1 „	+	0·09 „	+	0·02 „	+	0·3 „	+	0·48	=	+ + +
(10)	1 „	+	0·09 „	+	0 „	+	0·3 „	+	0·5	=	0

1½ hour at 37° C.

Here it will be seen that, in spite of the great excess of heated immune serum (of which 2 c.c.+2 c.c. 1 per cent. ox blood corpuscles mixed and incubated gave no hæmolysis in one and a half hour), there is no change in the complement content after injection, thus showing that the large excess of immune body does not affect an accurate comparative estimation of the amount of complement.

(2) The following experiment shows the great lowering of the M.H.D. of complement when excess of heated immune serum is added :—

Complement Undiluted.

(1) *Without Excess of Heated Immune Serum.*

	1 per cent. O.B.C.		H.I.S. 1 in 10.		Saline.		Compt.		Saline.		
(1)	1 c.c.	+	0·08 c.c.	+	0·3 c.c.	+	0·5	+	0	=	C.
(2)	1 „	+	0·08 „	+	0·3 „	+	0·4	+	0·1	=	C.
(3)	1 „	+	0·08 „	+	0·3 „	+	0·3	+	0·2	=	C.
(4)	1 „	+	0·08 „	+	0·3 „	+	0·2	+	0·3	=	C.
(5)	1 „	+	0·08 „	+	0·3 „	+	0·1	+	0·4	=	+ + +
(6)	1 „	+	0·08 „	+	0·3 „	+	0·08	+	0·42	=	+ +
(7)	1 „	+	0·08 „	+	0·3 „	+	0·06	+	0·44	=	+ ?
(8)	1 „	+	0·08 „	+	0·3 „	+	0·03	+	0·47	=	0
(9)	1 „	+	0·08 „	+	0·3 „	+	0·01	+	0·49	=	0
(10)	1 „	+	0·08 „	+	0·3 „	+	0	+	0·5	=	0

1½ hour at 37° C.

(2) *With Excess of Heated Immune Serum.*

	1 per cent. O.B.C.		H.I.S. 1 in 10.		H.I.S.		Compt.		Saline.		
(1)	1 c.c.	+	0·08 c.c.	+	0·3 c.c.	+	0·5	+	0	=	C.
(2)	1 „	+	0·08 „	+	0·3 „	+	0·4	+	0·1	=	C.
(3)	1 „	+	0·08 „	+	0·3 „	+	0·3	+	0·2	=	C.
(4)	1 „	+	0·08 „	+	0·3 „	+	0·2	+	0·3	=	C.
(5)	1 „	+	0·08 „	+	0·3 „	+	0·1	+	0·4	=	C
(6)	1 „	+	0·08 „	+	0·3 „	+	0·08	+	0·42	=	C.
(7)	1 „	+	0·08 „	+	0·3 „	+	0·06	+	0·44	=	C.
(8)	1 „	+	0·08 „	+	0·3 „	+	0·03	+	0·47	=	C.
(9)	1 „	+	0·08 „	+	0·3 „	+	0·01	+	0·49	=	+ ?
(10)	1 „	+	0·08 „	+	0·3 „	+	0	+	0·5	=	0

1½ hour at 37° C.

One per cent. ox blood corpuscles suspension was used; M.H.D. of heated immune serum used, for 1 c.c. 1 per cent. suspension of ox blood corpuscles = 0·08 of a 1 in 10 dilution. This dilution was used for sensitisation of the corpuscles; while the full strength serum in doses of 0·3 c.c. was added to give a great excess of immune body.

Here it will be seen that the addition of 0·3 c.c. heated immune serum (H.I.S.) lowered the M.H.D. of the complement from 0·2 c.c. of 0·03 c.c. The heated immune serum itself contained no complement, for 1 c.c. of it + 1 c.c. 1 per cent. suspension of ox blood corpuscles incubated at 37° C. for one and a half hour showed no hæmolysis.

These experiments may be divided into two sets—the first where sensitised, saturated, and non-sensitised corpuscles were injected into fresh rabbits, and the second where sensitised (saturated) and non-sensitised corpuscles were injected into immune rabbits. In the first set it will be seen that the injection of corpuscles, sensitised, saturated, or nonsensitised intravenously in sufficient quantity to use up practically all the complement in the animal's blood, if the animal were bled to death and the test made with the serum obtained *in vitro*, has produced absolutely no effect in the complement content of the serum, as shown by the M.H.D. of the complement before and after injection being the same. This has happened in spite of the fact that where sensitised and saturated corpuscles were injected, hæmolysis *in vivo* did take place, as shown by the intense hæmoglobinuria and the intense, deep red colour of the serum obtained after injection. Where non-sensitised corpuscles were used for injection no effect was produced, the urine not being tinted, and the serum was only coloured to an extremely small degree. This last might have arisen from hæmoglobin present in the serum injected due to the clot being manipulated to hasten separation.

The results obtained from the sensitised and saturated corpuscle injections may indicate that complement is not a definite limited quantity in the plasma of the blood, that it is not free in the plasma, but that it is given off from contact of its source with certain foreign bodies (in these experiments, the sensitised corpuscles injected), and that it is extremely rapidly regenerated. They seem to show that the tissues have a great reserve power for the production of complement, a condition apparently inconsistent with the view that the complement is a limited quantity free in the plasma. Complement may be present in the plasma, but there is no direct proof that it is, and the characteristics of complement action have only been definitely determined in serum *after clotting*. The introduction of a sensitised foreign body (*i.e.* possibility of starting the initial stages of clotting) into the blood stream has been shown above to bring about a phenomenon suggestive of complement action (namely, the hæmoglobin tinted serum and urine). In spite of this no fall in complement was

(as has been shown) detected between the bleedings. To account for the complement reading remaining the same, it seems necessary to suppose that fresh complement passed into the circulation during the experiment. Such considerations naturally raise the question of the existence of complement in a free state, and suggest a vital relation between a living cell and a foreign body.

The above experiments also show the necessity for the presence of both complement and immune body for hæmolysis *in vivo*; for when unsensitised corpuscles were injected into a fresh rabbit there was practically no hæmolysis to be noted; in later experiments, performed with special care to determine this point—especially to allow absolutely spontaneous separation of the clot from the serum, the serum after injection showed not the slightest trace of hæmolysis.

In the Series II., where sensitised (saturated) and non-sensitised corpuscles were injected into an immune rabbit, the effect is different; but here again a vital reaction of the tissues in the production of complement may be suggested. In all the cases where the corpuscles were injected into an immune rabbit the specific action of the complement was much less after injection than before injection, as shown by the same moiety of serum after injection producing much less hæmolysis *in vitro* than before injection. This lowering of the complement action has taken place in spite of the fact that the complementing power (not necessarily the *amount* of complement, *vide* p. 528) of an immune rabbit's serum is much greater than that of a fresh rabbit, and that consequently the injection of equal quantities of blood corpuscles into immune and fresh rabbits of the same blood volume should, instead of causing a diminution of complementing power in the immune rabbit, rather favour the complement remaining at what it was before injection. An example may make this clear. In Series II., Experiment 1, a fresh doe rabbit, weight 1460 grms., was injected with 2·8 c.c. saturated ox blood corpuscles. The complement remained the same before and after injection. Here, if we take 0·6 c.c. (see Experiment) as the M.H.D. for 2 c.c. 1 per cent. ox blood corpuscle suspension, then the 2·8 c.c. should have used up 84 c.c. of complement; but the total amount of complement present in the animal's body was only about 38·6 c.c.: yet in spite of this the complement content remained the same before and after injection. On the other hand, in Series IV., Experiment 1, where an immune buck rabbit, weight 2150 grms., was injected with 3 c.c. fully saturated ox blood corpuscles, the complement was very greatly diminished in the serum obtained after injection. Here, if we take 0·15 c.c. (see Experiment) as the M.H.D. of complement for 2 c.c. 1 per cent. suspension of ox blood corpuscles before injection, then the 3 c.c. ox blood corpuscles ought to have used up only 22·5 c.c. of the complement; while the amount of complement present in the whole animal's body amounted to 52 c.c. This might be explained by

supposing some change to occur in the vital production of complement in an immune rabbit as the result of its immunity, allowing complement to be more easily dissociated from the cells with less reserve power in the body for production of a large quantity of this substance; alternatively, the injection of the corpuscles might so derange the vital functions and upset metabolism that for the moment the organism does not respond, and the complement determination after injection would thus show a diminution. In support of this latter view is the fact that all the immune rabbits injected were severely ill, three of them practically dying before the expiry of the one and a half hour limit—one actually dying in five minutes. This may have been due to minute emboli of red cell stromata or agglutinated red cells; and evidence of infarction was seen in several of the rabbits; but there is a possibility of a breakdown of the whole mechanism of the body, comparable to the breakdown occurring in horses highly immunised against diphtheria-toxin on the injection of a further dose.[1] However the breakdown occurs, the fact that the animals were extremely ill shows that this might have something to do with the diminution of complement.

There are some facts which point, however, to there being a gradual change in complement production in immune rabbits as the result of the immunisation. Of the six fresh rabbits injected, only one showed any sign of illness. It was very ill for the first half-hour, and looked as if it would die. Here the cause of the illness was undoubtedly minute flocculi visible to the naked eye in the blood suspension used for injection. In spite of this illness there was no diminution of complement in the serum obtained after injection. Again, the immune rabbit which had been immunised for about six months (Experiment 1, Series V.), and which died in five minutes, showed of all the rabbits the greatest diminution in complement in the serum obtained after injection. This might have been due to lack of time for regeneration, but the marked diminution is significant, especially when taken in connection with rabbit of Experiment 2, Series V. This rabbit had only been immunised for about three weeks. After injection it was very ill, but recovered and looked quite like living when the time limit (one and a half hour) expired. Here the diminution in complement was very slight indeed in the serumof the injection. Whatever view is taken, as to the immediate cause after diminution of complement,—whether there is a so to speak momentary lowering of vital reaction caused by the flooding in of the corpuscles, or a more prolonged moulding action causing complement to be more easily dissociated and less easily regenerated, as in the case of the fresh rabbits,—the supposition of a vital reaction of some issue in relation to a foreign body causing that tissue to give off complement may be entertained.

[1] This point is being further investigated at present.

Conclusion.

The injection intravenously of unsensitised, sensitised, or saturated ox blood corpuscles into fresh rabbits produces no diminution of complement; whereas the injection intravenously of unsensitised or sensitised ox blood corpuscles into immune rabbits (*v.* ox blood corpuscles) produces a marked diminution in complement.

I wish to express my indebtedness to Dr. Ritchie, Superintendent of the Royal College of Physicians, Laboratory, Edinburgh, for suggesting the line of research, and for his help and criticism. I wish also to thank the Laboratory Committee for the facilities afforded in their laboratory for carrying on the work. This work was done while holding the Ernest Hart Research Scholarship of the British Medical Association, and on a grant for research from the Carnegie Trust.

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THE FATE OF HEN CORPUSCLES WHEN INJECTED INTRAVENOUSLY INTO RABBITS, WITH SOME CONSIDERATIONS AS TO THE SEAT OF THE FORMATION OF IMMUNE BODY.[1]

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This research was undertaken at the suggestion of Dr. James Ritchie, the object being to determine, if possible, the ultimate fate of hen corpuscles when injected into the blood stream of rabbits. The expenses of the research were defrayed by a grant from the Carnegie Trust, for which I wish to express my cordial thanks to the Trustees.

Levaditi (1901[1]) injected intravenously into normal and immunised animals cholera vibrios, and found that in the immunised animals so injected the vibrios were not transformed into granules in the circulating plasma. The transformation took place only in the polymorphs circulating in the blood. He found the granular degeneration of the vibrios taking place *in the organs* extracellularly, however, and this he attributes to complement being set at liberty from the phagocytes accumulated in the interior of these organs. In 1905, in continuation of this work, Levaditi published a paper[2] in which he investigated the fate of pigeon corpuscles when injected intravenously into guinea-pigs. Guinea-pigs' serum, normally, is lytic to pigeons' red blood corpuscles; according to Levaditi, 0·5 c.c. completely dissolving a drop of pigeon's blood. He injected into the jugular veins of fresh guinea-pigs 1 to 1·2 c.c. of a thick emulsion of pigeon corpuscles. The guinea-pigs bore such an injection quite well. When, however, such an injection was practised on guinea-pigs which had been immunised against pigeon corpuscles by previous injections, they were seized with great dyspnœa and extreme collapse. Respiratory difficulties predominated. The difference of effect produced in the fresh and immunised guinea-pigs by injection he attributed to a different mechanism of destruction of the pigeon blood corpuscles in the two cases. When the fresh guinea-pigs are injected, the destruction takes place by *an extremely rapid and energetic phagocytosis in the interior of the spleen*, while in the immunised animals the destruction takes place extracellularly, and phagocytosis is hardly to be observed at all. He found further that if he examined the blood of fresh guinea-pigs five, thirty, forty, and one hundred minutes after an injection with pigeon blood the pigeon corpuscles persisted unaltered.

[1] Received December 9, 1909.

[2] "Le sort des hématies nuclées introduites dans la circulation general des animaux neufs ou immunisés a l'aide de ces hématies," C. Levaditi, Bucarest, Imprimerie de l'état, 1905.

They agglutinated round masses of platelets and were lying in intimate contact with the polynuclear leucocytes. At no time could he see any evidence of extracellular solution of the pigeon corpuscles. He further found, if he killed the animal at a time when the pigeon corpuscles had disappeared from the general circulation and carefully examined the spleen, that rapidly, sometimes in less than forty minutes, the macrophages of that organ were filled with the pigeon corpuscles. He found that the phagocyted pigeon corpuscles were much more numerous than the free ones, and that the englobed corpuscles rapidly showed signs of intraprotoplasmic digestion. He found that the liver only of all the other organs showed any signs of phagocytosis of the pigeon corpuscles. Here the phagocytes were the Kupffer cells. No pigeon corpuscles, phagocytosed or free, were found by him in the lymphatic glands or in the lungs.

This paper of Levaditi's, as well as his previous one, was adduced as evidence of the non-existence of complement free in the circulating plasma, and in support of its origin entirely from leucocytes. Thus an extremely wide question is opened up, and I shall confine myself here to the points specifically raised by Levaditi, and to a criticism of the methods by which he arrived at such far-reaching conclusions. In the first place, he dismisses in a footnote the behaviour of normal guinea-pigs' serum towards pigeon corpuscles, by saying that 0·5 c.c. fresh guinea-pig serum dissolves, completely, a drop of pigeon blood. No time is mentioned, no temperature of reaction considered, the pigeon corpuscles are left in contact with their serum (*i.e.* unwashed), a drop is a very variable quantity, and no special consideration is given to the question of whether or not every guinea-pig's serum has the same lytic action *in vitro* towards pigeon corpuscles. The burden of these criticisms is that, as the question to be solved is the nature and means of lysis of pigeon corpuscles *in vivo*, care should have been taken to determine roughly, in each experiment, whether the serum of the animal experimented on was lytic *in vitro*. There is no record of this having been done, and the omission may explain the marked persistence of pigeon corpuscles in the circulating blood of fresh guinea-pigs which Levaditi found—sometimes for over one hour and a half. Again, such a neglect cuts at the root of his criterion of the presence or absence of complement in the circulating blood. He adopts in fresh guinea-pigs destruction by phagocytosis in the blood stream and absence of any destruction extracellularly as proof that complement does not exist free in the circulating plasma. Yet it may be that the serum of the guinea-pig which he has experimented on, and from observations on which he has drawn such conclusions, was non-lytic to pigeon corpuscles *in vitro*; so that there is no proof that the phagocytosis which he finds *in vivo* and the lysis *in vitro* are one and the same reaction. Further, Levaditi, in accounting for the difference of reaction to injection in fresh and immunised guinea-pigs—no disturbance in the one case and violent dyspnœa in the other—states that in the case of the fresh guinea-pigs there is no extracellular destruction, while in the immunised guinea-pigs there is extracellular

destruction and hardly any intracellular. But why has the mere process of immunisation transformed a wholly intracellular process into an almost completely extracellular process, which Levaditi admits? The question becomes too involved here, and admits of the play of unlimited sophistry, and I shall confine myself to a consideration of one definite statement of Levaditi's on which the whole argument hangs—that of the occurrence of the phagocytosis in fresh animals, which he states is all-important in *in vivo* lysis of foreign corpuscles. It is immaterial to a consideration of the main question what animals and what nucleated corpuscles are used, so long as the serum of the animal lyses *in vitro* the nucleated corpuscles employed. For this reason, and as being more easy to work with, I have used rabbits treated with hen corpuscles, ascertaining in each experiment whether the serum of the rabbit used actually hæmolysed *in vitro* the hen corpuscles used. So that if I find that in the rabbit, whose serum *in vitro* lyses hen corpuscles, there is absolutely no sign of phagocytosis when it is injected intravenously with hen corpuscles, I am entitled to say, using his criterion for the existence or not of complement in the circulating plasma, that although Levaditi's actual experimental findings may be strictly as he says, his general deduction that complement does not exist free in the circulating plasma, but is formed from the phagolysis of the leucocytes, is not correct, for here in the case of the rabbit and hen corpuscles is a marked exception. I hope also from these experiments to carry my opposition to his deduction further, and to bring forward some evidence from these experiments to show that complement does exist free in the plasma. That it is or is not formed by the leucocytes cannot be determined by such an experiment, and where it is formed, if not formed by the leucocytes, is beyond the scope of the present paper.

It may be stated that my work was commenced and completed before I had access to Levaditi's paper,[1] so that it may be looked on as an independent investigation of the subject.

In the main the corpuscles used for injection were obtained by bleeding hens from the carotid, defibrinating the blood, washing the corpuscles free from serum, and then filtering through fine wire gauze to remove masses of clot. The rabbits were bled from the ear vein to obtain a sample of the serum for estimation of its hæmolytic power to the hen corpuscles. They were then injected intravenously with 2 c.c. of the washed hen corpuscles diluted with saline, and killed at various intervals after the injection. At the post-mortem examination the various organs were at once fixed in various fixatives, *e.g.* 5 per cent. formalin, saturated $HgCl_2$, and alcohol. The urine was carefully examined for presence or absence of hæmoglobin and the deposit for blood corpuscles—rabbit or bird—to exclude the possibility of bleed-

[1] I am indebted to Dr. Cramer for kindly lending me Levaditi's paper and for many other references.

ing from the kidney or passage of the bird's corpuscles through the kidney into the urine. In some cases films were made from the blood of the rabbits at short intervals after the injection; such films were examined for the presence of hen corpuscles and for possible phagocytosis.

Experiment 1.

In this experiment six rabbits were used: Rabbits 1, 2, 3, 4, 5, 6. They were all bled 10 c.c. from the ear vein. This blood was allowed to clot and the serum to separate. Next day the sera were tested for their lytic power on the hen corpuscles to be used for injection. The hen corpuscles were washed six times with normal (0·85 per cent.) saline, and a 5 per cent. suspension was made.

The protocol of Rabbit 1 was as follows:—

Five per Cent. Suspension of Hen's Corpuscles.	Rabbit Serum.	Saline.		
1 1 c.c. . . .	+0·1	+1·4	One and a half hour at 37° C.	=0
2 ,, . . .	+0·2	+1·3		=+
3 ,, . . .	+0·4	+1·1		=+
4 ,, . . .	+0·8	+0·7		=+++
5 ,, . . .	+1·0	+0·5		=++++
6 ,, . . .	+1·5	+0		=C.
7 ,, . . .	+0·0	+1·5		=0

C. = complete hæmolysis.
++++ = nearly complete hæmolysis.
+++ = marked hæmolysis.
++ = good amount.
+ = trace.
0 = none.

The sera of the other rabbits were tested in the same way and the results were briefly:—

Rabbit 2.	Rabbit 3.	Rabbit 4.	Rabbit 5.	Rabbit 6.
1=0	1=0	1=+(?)	1=+	1=0
2=0	2=0	2=+	2=+	2=0
3=0	3=0	3=+	3=+	3=+(?)
4=0	4=0	4=+++	4=+++	4=+
5=+(?)	5=+	5=++++	5=++++	5=+
6=+	6=+	6=++++	6=++++	6=+
7=0	7=0	7=0	7=0	7=0

++++ = nearly complete hæmolysis.
+++ = marked hæmolysis.
+ = trace.
0 = none.

The rabbits are thus divided into two groups. Rabbits 1, 4, and 5 with fairly strong lytic sera to hen corpuscles, and Rabbits 2, 3, and 6 with sera weakly lytic to hen corpuscles. Rabbits 1, 4, and 5 were kept as controls, and 2, 3, and 6 were inoculated intravenously with the washed hen corpuscles. In this experiment too, taking into consideration the changes observable in

the organs, especially in the liver at various stages of digestion, the rabbits were all kept in the same cage, subject to the same conditions, for a week previous to injection. They were fed regularly at 9.45 every morning on corn, bran, and cabbage-blades. By keeping them rigidly under the same conditions, and by the use of the controls, the marked glycogenic changes in the liver due to feeding were appreciated as such. Otherwise these might have been put down—so markedly necrotic looking the liver cells were—to a toxic action of some constituent of the hen corpuscles.

Rabbit 2 (weakly lytic) and Control Rabbit 1 (lytic).—Rabbit 2 had been fed at 9.45, and was injected at 11.5 intravenously (by ear vein) with 2 c.c. hen corpuscles washed six times + 2 c.c. (0·85 per cent.) saline. Blood films were made from the ear vein of the other ear at ten minute intervals until the animal was killed. It showed no signs of discomfort after injection, was kept without food, and killed at 12.5,—one hour after injection. There was *no* hæmoglobin in the urine, and no cell elements were found in the deposit. The bladder was not distended; the urine was very clouded; the gall bladder was not distended; the spleen was not enlarged; cestode parasites were present in the peritoneal cavity. There were no hæmorrhages visible on any of the organ surfaces or in the lungs. Kidney, spleen, liver, and lung were fixed in saturated $HgCl_2$, alcohol, and formalin.

Bone marrow smears were made.

Examination of the films, smears, and sections of this rabbit gave the following results:—

Films.

Film made at	11.15,—10 minutes	after injection, . .	Hen corpuscles present.
,, ,,	11.25,—20 ,,	,, . . .	A few present.
,, ,,	11.35,—30 ,,	,, . . .	None found.
,, ,,	11.45,—40 ,,	,, . . .	None found.
,, ,,	12.0, —55 ,,	,, . . .	None found.

Smear of the bone marrow: no hen corpuscles could be distinguished.

Sections.

Kidney.—No hen corpuscles seen in the kidney—either in the vessels or in the tubes. There were no hæmorrhages. *Spleen.*—Showed a few hen corpuscles, but not a trace of phagocytosis,—about one hen corpuscle was found in every three fields of the high power ($\frac{1}{6}$). *Lung.*—No hen corpuscles were found in the lung. *Liver.*—Hen corpuscles were found blocking the capillaries of the liver,—in one field of the high power ($\frac{1}{6}$) as many as six clumps of hen corpuscles were found, each clump containing three or four hen corpuscles. No phagocytosis was observed in this case.

Rabbit 1 had been kept under the same conditions as to cage, feeding, etc. as Rabbit 2. It was killed and its organs fixed at the same time as Rabbit 2. No hæmoglobin was found in its urine. The bladder was much distended. The gall bladder was not distended. Spleen was not enlarged; parasites were present in the peritoneal cavity. Sections were cut of the various organs, and the only purpose they served was to demonstrate the extreme variability of the appearances of the liver cells in the rabbit. In this case these cells showed a faintly staining nucleus in the centre, a thickened polygonal rim of dull-staining protoplasm, and an intermediate large zone which was empty. The cell was much swollen, and looked as if it were necrosed. Such findings in a presumably normal rabbit prevented one from concluding that the minor degree of the change found in Rabbit 2 was a toxic one. The change is simply due to glycogenic transformation, but it is very confusing, and liable to lead one astray.

Rabbit 3 (weakly lytic) and Control 4 (lytic).—Rabbit 3 had been fed at 9.45 a.m., and was injected at 11.8 intravenously (by ear vein) with 2 c.c. hen corpuscles (washed six times) + 2 c.c. (0·85 per cent.) saline. The rabbit was kept without food till killed. It showed no signs of discomfort after injection. Blood films were made from the ear vein of the other ear at 8 a.m., 12.30, 1, 3, and 4 p.m. The rabbit was killed at 4.10,—five hours after injection. There was no hæmoglobin in the urine; parasites were present in the peritoneum. Gall bladder was not distended. The spleen was not enlarged. There were no hæmorrhages to be observed on the organ surfaces or in the lung. Specimens of the kidney, spleen, liver, and lung were fixed as before.

Examination of the films and sections of this rabbit gave the following results:—

Blood Films.

No hen corpuscles found in any of the films.

Sections.

Kidney.—Showed no hen corpuscles or hæmorrhages. *Spleen.*—No hen corpuscles seen. *Liver.*—Showed hen corpuscles present in fairly large numbers. They were markedly necrotic; no phagocytosis.

Rabbit 4 (Control).—Had been subjected to the same conditions as Rabbit 3, and was killed at 4.20. There was hæmoglobin in urine; the bladder was distended. Parasites were present in the peritoneum; examination of the sections served the same purpose as in Rabbit 1,—prevented a wrong interpretation being put on the appearances in the liver cells.

Rabbits 6 (weakly lytic) and 5 (Control) (lytic).—Rabbit 6 had been fed at 9.45, and was injected at 11.11 intravenously (by ear vein) with 2 c.c. hen corpuscles (washed six times) + 2 c.c. (0·85 per cent.) saline. It showed no signs of distress after injection; a blood film was taken from its ear vein next morning at 10.45. The rabbit was killed at 11 o'clock next morning, practically twenty-four hours after injection. Food was withheld from it on the morning when it was killed. There was no hæmoglobin in its urine; bladder was slightly distended; gall bladder was not distended; no hæmorrhages seen anywhere; spleen was slightly enlarged. Specimens from the kidney, liver, spleen, and lung were fixed as before.

Examination of the films and sections gave the following results:—

Films.

No hen corpuscles seen.

Sections.

Kidney.—No hen corpuscles or hæmorrhages seen. *Spleen.*—No hen corpuscles seen. *Liver.*—No hen corpuscles seen, and liver cells extremely necrotic looking, swollen, and vacuolated, as they appeared also in the control Rabbit 5, which had no other point of interest.

Experiment 2.

In this experiment two rabbits were taken, Rabbits 7 and 8. Ten c.c. blood were removed by the ear vein. The blood was allowed to clot and the serum to separate. The sera were then tested for their lytic power against the hen corpuscles to be used for injection. The hen corpuscles were washed six times with saline to free them from serum, and a 5 per cent. suspension was used for the test.

Rabbit 7.

Five per Cent. Suspension of Hen Corpuscles.	Rabbit Serum.	Saline.		
1 1 c.c.	+0·1	+1·4	One and a half hour at 37° C.	=+
2 ,,	+0·2	+1·3		=+
3 ,,	+0·4	+1·1		=+ +
4 ,,	+0·8	+0·7		=+ + +
5 ,,	+1·0	+0·5		=+ + + +
6 ,,	+1·5	+0		=C.
7 ,,	+0	+1·5		=0

Rabbit 8.

1 1 c.c.	+0·1	+1·4	One and a half hour at 37° C.	=0
2 ,,	+0·2	+1·3		=+
3 ,,	+0·4	+1·1		=+
4 ,,	+0·8	+0·7		=+ + +
5 ,,	+1·0	+0·5		=+ + + +
6 ,,	+1·5	+0		=C.
7 ,,	+0	+1·5		=0

C. = complete hæmolysis. + + = good amount.
+ + + + = nearly complete hæmolysis. + = trace.
+ + + = marked hæmolysis. 0 = none.

Both these rabbits had therefore fairly good lytic sera.

Rabbit 7 (buck, weight 2390 grms.).—Fed at 9.20 with cabbage, corn, and bran. Was injected intravenously (by ear vein) at 10.35 with 2 c.c. hen corpuscles (washed six times) + 2 c.c. saline.

Rabbit 8 (weight 1625 grms.).—Fed at 9.20 with cabbage, bran, and corn. Injected intravenously into ear vein at 10.35 with 2 c.c. hen corpuscles (washed six times) + 2 c.c. saline. Both rabbits were catheterised at end of twenty minutes. *Hæmoglobin was found in the urine of both.* Blood was drawn from the ears of both at the end of twenty-five minutes. The serum of Rabbit 7 was distinctly hæmoglobin tinted, while the serum of Rabbit 8 was darkly hæmoglobin tinted Five and a half hours after injection the urine of Rabbit 7 contained no hæmoglobin, while the serum of Rabbit 8 was still darkly hæmoglobin tinted.

Experiment 3.

In this experiment nine rabbits were used. All had good lytic sera towards hen corpuscles *in vitro.*

Rabbit 9 (doe).—Was injected intravenously (by ear vein) with 2 c.c. hen corpuscles (washed six times) + 2 c.c. normal saline at 3.42. It was killed at 3.52,—ten minutes after injection. There was no hæmoglobin in the urine. There were parasites in the abdomen. The spleen was very large; the gall bladder was not distended. Specimens from the spleen, liver, lung, and kidney were fixed as before.

Examination of the sections gave the following results :—

Kidney.—No hen corpuscles and no hæmorrhages found. *Spleen.*—Pulp engorged with blood and spaces greatly distended. (This, taken into consideration with the great increase in size of the organ, may be explained by a

dilatation of the spleen, acute in type, to make room for the injected material.) Hen corpuscles were seen in fairly large numbers; no phagocytosis was seen. *Liver.*—Hen corpuscles present in very great numbers; no phagocytosis seen. *Lung.*—No hen corpuscles seen.

Rabbit 10 (doe).—Was injected intravenously (by ear vein) at 3.45 with 2 c.c. hen corpuscles (washed six times) + 2 c.c. saline. It was killed at 4.5, twenty minutes after injection. There was no hæmoglobin in the urine. There were parasites in the abdomen. The spleen was not enlarged; gall bladder was not distended; specimens from the spleen, liver, kidney, and lung were fixed as before.

Examination of the sections gave the following results:—

Kidney.—No hen corpuscles and no hæmorrhages seen. *Spleen.*—Large number of hen corpuscles seen; no phagocytosis. *Liver.*—Very large number of hen corpuscles seen; some phagocytosis of the hen corpuscles by Kupffer's cell. *Lung.*—No hen corpuscles seen in lung.

Rabbit 11 (big grey doe).—Injected intravenously (by ear vein) at 11 o'clock with 2 c.c. hen corpuscles (washed six times) + 2 c.c. saline. There was no distress after injection. The rabbit was killed at 11.30,—half an hour after injection. The bladder was distended with darkly hæmoglobin-tinted urine. No hen or rabbit corpuscles were found in the deposit from the urine; smears were made from the femur and rib marrow. Specimens from the lung, liver, spleen, kidney, and bone marrow were fixed as before. The spleen was not enlarged. The gall bladder was not distended.

Examination of the films and sections gave the following results:—

Films.

In bone marrow films no hen corpuscles were observed.

Sections.

Kidney.—No hen corpuscles and no hæmorrhages were observed. *Spleen.*—The hen corpuscles were present in small numbers, and there was no trace of phagocytosis. *Liver.*—The hen corpuscles were present in large numbers, and there were a very few instances of phagocytosis by the Kupffer cells. *Lung.*—No hen corpuscles seen.

Rabbit 12 (doe).—Was injected intravenously into ear vein with 2 c.c. hen corpuscles (washed six times) + 2 c.c saline at 11.5. There was no sign of distress. It was killed at 12.5,—one hour after injection. The bladder was distended with dark hæmoglobin coloured urine. No hen or rabbit blood corpuscles were found in the deposit from the urine. There were parasitic cysts in the abdomen. Specimens from the liver, lung, spleen, and kidney were fixed as before. Bone marrow smears were made. The spleen was not enlarged. The gall bladder was not distended.

Examination of the films and sections gave the following results:—

Films.

No hen corpuscles seen in the bone-marrow films.

Sections.

Kidney.—No hæmorrhages and no hen corpuscles seen. *Spleen.*—Hen corpuscles hardly to be found; no phagocytosis. *Liver.*—Hen corpuscles found in large numbers; a few instances of phagocytosis by Kupffer's cells. *Lung.*—No hen corpuscles seen.

Rabbit 13.—Was injected intravenously with 2 c.c. hen corpuscles (washed six times) + 2 c.c. saline at 2.15. There was no sign of distress. It was killed at 3.15,—one and a half hours after injection. The bladder was distended with deeply hæmoglobin-stained urine. No hen or rabbit blood corpuscles found

in the deposit from the urine. Specimens from liver, spleen, and kidney fixed as before. Films were made from bone marrow.

Examination of the films and sections gave the following results:—

Films.

Films of bone marrow: no hen corpuscles seen.

Sections.

Kidney.—No hæmorrhages and no hen corpuscles. *Spleen.*—No hen corpuscles seen. *Liver.*—Hen corpuscles present in small numbers. Phagocytosis doubtfully present.

Rabbit 14 (buck, small brown grey, with large ears).—Injected at 11.10 with 2 c.c. hen corpuscles (washed six times) + 2 c.c. saline intravenously (by ear vein). There was no sign of distress. It was killed at 1.10,—two hours after injection. The bladder was distended with hæmoglobin-tinted urine. No hen or rabbit blood corpuscles in the deposit. There were no parasites in the peritoneum. Liver, kidney, spleen, and lung fixed as before.

Examination of the sections gave the following results:—

Kidney.—No hen corpuscles and no hæmorrhages. *Spleen.*—No hen corpuscles and no phagocytosis. *Liver.*—Hen corpuscles present in small numbers—fused. Phagocytosis not observed. (In those rabbits killed at later periods it was difficult in the liver to observe the hen corpuscles owing to the very great swelling of the liver cells with glycogen.) *Lung.*—No hen corpuscles seen.

Rabbit 15 (buck, white, black nose).—Was injected intravenously by ear vein at 11.12 with 2 c.c. hen corpuscles + 2 c.c. saline as before. There was no sign of distress. The rabbit was killed at 4.12,—five hours after injection. There was no hæmoglobin in the urine. Specimens from the liver, spleen, kidney, and lung were fixed as before.

Examination of the sections gave the following results:—

Kidney.—No hen corpuscles and no hæmorrhages. *Spleen.*—No hen corpuscles and no phagocytosis. *Liver.*—An occasional hen corpuscle seen. *Lung.*—No hen corpuscles seen.

Rabbit 16 (grey buck).—Was injected intravenously by ear vein as before at 11.15. There was no sign of distress. It was killed next day at 9.30,—twenty-three hours after injection. There was no hæmoglobin in the urine. There were no parasites in the abdomen. Specimens were fixed as before from the liver, lung, spleen, and kidney.

Examination of the sections gave the following results:—

In the kidney, spleen, liver, and kidney no hen corpuscles were seen, and there was no hæmorrhage.

Rabbit 17 (doe).—Injected as before at 11.20, killed forty-six hours later. No hæmoglobin in urine. No parasites in abdomen. Specimens fixed as before. No hen corpuscles seen in any of the organs on microscopical examination.

In these experiments the most notable thing was the almost entire absence of phagocytosis. The only place where I observed phagocytosis was in the Kupffer cells of the liver, and then to a very limited extent. Nevertheless hæmolysis of the injected hen corpuscles took place *in vivo*, its presence being determined by drawing off samples of blood, catheterising the rabbits and examining the bladder at the post-mortem examinations. Where the serum of the rabbits

was actively hæmolytic to the hen corpuscles *in vitro*, it was actively hæmolytic *in vivo*, as evidenced by blood-stained serum and blood-stained urine being obtained in these cases as early as twenty minutes after the injection. This hæmolytic activity was also shown by the scantiness of the hen corpuscles in the organs of these rabbits as compared with their frequency in the organs of rabbits with a serum weakly lytic *in vitro*. In the latter rabbits, after an hour, the liver capillaries were found choked with plugs of the hen corpuscles. Films made from the blood of such rabbits showed that hen corpuscles were absent from the general circulation in about thirty minutes. In both series of rabbits the phenomenon chiefly noticeable was the accumulation of the hen corpuscles in the liver, where they could be found in larger quantities and for much longer time than in any of the other organs. They could be found in the spleen at the end of an hour with difficulty, in marked contrast to the ease with which they could be found in the liver. In no other organ could I find them even ten minutes after injection. Again, the almost entire absence of phagocytosis was a noticeable feature, in contrast with what Levaditi found when he injected guinea-pigs with pigeon corpuscles. I only observed this phenomenon about twice altogether—in the liver of Rabbit 11 (Experiment 3). That was half an hour after injection. On the contrary, I did not observe it in Rabbit 2 (Experiment 1) one hour after injection, although the liver was crammed with hen corpuscles. Nor did I observe any evidence of phagocytosis by the leucocytes in the blood stream of Rabbits 2, 3, or 6 (Experiment 1). In spite of the absence of phagocytosis, which according to Levaditi is the cause of the lysis of the pigeon corpuscles observed in his experiments, hen corpuscles when injected intravenously into a rabbit with strongly lytic serum *in vitro* were rapidly lysed even in twenty minutes, as evidenced by the deep hæmoglobin-tinted urine and serum. Where the serum was weakly lytic *in vitro* there was a corresponding weak lytic action *in vivo*, as shown by the absence of hæmoglobin in the urine, and by the persistence of the hen corpuscles in much larger numbers and for a much longer time in the liver. The rapidity of the lysis *in vivo* —the urine and serum appearing darkly hæmoglobin tinted in twenty minutes—is also against phagocytosis being an explanation of the lysis. Levaditi further states that when his guinea-pigs had been immunised against pigeon corpuscles, and were then injected intravenously, phagocytosis was not the cause of the rapid lysis, but the rapid lysis took place in the blood extracellularly.

It is a dictum of Metchnikoff and his school, to which Levaditi belongs, that complement is set free in the blood stream by the "phagolysis" incident on the damaging effects on the leucocytes of the blood by an intravenous injection of serum, blood corpuscles, etc. It is also one of their beliefs that this phagolysis can be prevented by a previous injection. Why, then, is not the complement set free in

the blood stream of fresh guinea-pigs when these are injected with pigeon corpuscles, and why are immune guinea-pigs so liable to such an event when similarly injected?

Rabbit serum contains in many cases a lysin for hen corpuscles, which shows the feature of a compound lysin in that it can be inactivated by heating to 56° C. for half an hour. Whether this lysin can be reactivated or not by some other non-lytic serum I have not as yet determined, but the probability is (to judge from analogous normal sera instanced by Ehrlich) that it can be so complemented. The rapidity with which the reaction takes place *in vivo*, and the absence of phagocytosis, shows to my mind that the *in vitro* and *in vivo* reactions are the same. Granting that the lytic action is a compound one—due to complement and immune body (natural)—the question arises, can we deduce from these experiments any information as to the seat of origin of the complement? Taken by themselves the experiments now detailed give no clue, but taken in conjunction with results to be published later which confirm and amplify the work of Falloise and Gruber, they go to show that the leucocytes—of the blood, spleen, bone marrow, etc.—do not form complement. The other point brought out by the experiments—the accumulation in and sifting out by the liver of the hen corpuscles—raises the question of how far the liver may be the seat of origin of immune body. Where the rabbit serum is strongly lytic *in vitro*, the lysis *in vivo*, in my opinion, takes place in the general blood stream,—not by means of any particular organ. So that this "primary" lysis being due to already existing immune body can give no clue to the seat of origin of immune body.

When ox blood corpuscles are injected intravenously in large quantities in a rabbit which has been carefully chosen with regard to its serum not being normally lytic to ox corpuscles, no lysis takes place until about the third day, and then it takes place critically, the rabbit passing hæmoglobin in its urine in large quantities for several hours. Sachs first showed this, and my own experiments (to be published later) amply confirm it. It is obvious that one cannot watch histologically what is taking place during these three days in the ox corpuscles, owing to their resemblance to rabbit corpuscles. But if one uses hen corpuscles, and especially if one uses hen corpuscles in rabbits with sera weakly lytic to hen corpuscles, one can get to know something at least of what is going on. The main phenomenon observed, as mentioned above, is the accumulation of the hen corpuscles in the capillaries or sinusoids of the liver. There is a trifling amount of phagocytosis by the Kupffer cells, but it is extremely small in amount, and has only been observed by me in one rabbit (possessing normally lytic serum) thirty minutes after injection. I need not say that it was far too small in amount to cause even a fraction of the "primary" lysis. That the hen corpuscles are not

specially liable to stick in the capillaries of the liver by their size, etc., is shown by the results of Pontick and others, who found that foreign particles of minute size, such as cinnabar, chinese ink, carmine, etc., when injected intravenously, tended to accumulate more in the liver than in any other organ. That there is a mechanism in the liver for dealing with such particles may be presumed to exist when we consider the markedly phagocytic powers of the Kupffer cells and the fine intracellular channels which have been shown by Herring and Simpson (1906 [2]), among others, to communicate directly with the capillaries or sinusoids of the liver. Blood corpuscles (Herring and Simpson) ([2]) and pneumococci (Weber, 1909 [3]) have been found inside liver cells, and I myself have a specimen from the above experiments (Rabbit 11, Experiment 3) in which several expert microscopists have given it as their opinion that a liver cell contains two hen corpuscles.

As regards some of the further evidence that the liver may be the seat of formation of the immune body, one may consider its function as a digestive gland. Many observers have produced antibodies to specific antigens by feeding the animal on the antigen. I myself have produced a lysin, agglutinin, and precipitin to ox blood by feeding rabbits on ox blood (the full details of the experiments I shall publish later). Many authors too have shown that the dose of a poison is much less when injected by the ear vein than when injected by the mesenteric vein. This may mean either that the liver has a specific neutralising power for toxins which might be exaggerated into the production and giving off of antibodies, or that the poison was simply mechanically retained and diluted by the large volume of blood in the liver. Whichever interpretation is taken, one can at least draw the deduction from such experiments that the liver by retaining these toxins in its substance has a special opportunity of acting on them which might lead to the production of an antibody. Such experiments give ground for the belief, too, that not only particulate objects are arrested in the liver, but also substances soluble in the blood. Delezenne (1898 [4]) further showed, by perfusion of the liver, that this organ was the seat of formation of antithrombin. His experiments are not convincing, but they are cited for the bearing they have on the present subject.

Experiments to determine in a Direct Way if the Liver is the Seat of Formation of Immune Body.

Metchnikoff and Tarassevitch had investigated this question by making extracts of various organs in saline and comparing the lytic action of such organ extracts with one another and with the blood serum. They also used this method of detecting a lytic power in certain extracts of organs before it was present in others or in the

blood serum. By this means they determined that the spleen and lymphatic tissue of the body were the seat of origin of the immune body. At the outset there are several strong objections to such a method. There is no method of estimating, once the immune body has appeared in the serum, how much of a certain organ or tissue is to be taken as corresponding to a certain amount of blood or blood serum. Where the immune body has not appeared in the serum, one must carefully differentiate between a lysis produced by autolysis of organ extracts and a true lysis due to the action of complement and immune body. Conradi (1901[5]) showed that sterile autolysates of organs would lyse not only the blood corpuscles of foreign species, but the animal's own blood corpuscles. Tarassevitch (1902[6]) has not taken this into consideration, and indeed states that the organ extracts lysed quite well after being heated for half an hour at 56° C., a fact which at once differentiates this lysis from one due to complement and immune body. He found also that the presence of debris in the organ emulsion protected the lytic power when the emulsion was heated, showing further that this lytic action had nothing to do with a true complement and immune body reaction. I had already performed the experiments recorded below on the question of the liver being the seat of formation of immune body, and had found that it was impossible to arrive at any conclusion from the use of organ extracts owing to the absorption of complement by the organ debris, when on looking up the literature I found that among others Von Dungern (1900[7]) and Muir (1903[8]) had recorded the absorptive power of organ debris for complement. This is the strongest criticism that can be brought against Tarassevitch's results, for he did not recognise this fact, and, whatever the lysis he found was due to, it certainly was not due to the combination of complement and immune body.

Experiments 1 and 2.

Two rabbits were taken and their serum was ascertained to be free from normal lysin for ox blood corpuscles. They were injected intravenously with 2 c.c. washed ox blood corpuscles, and they were killed by bleeding from the carotid artery twenty-four hours after injection. The object of killing so early was to get the animal's serum before any immune body appeared in it,—immune body usually not appearing in the serum under these conditions until about the third day. It was hoped, however, that some evidence of the presence of this substance in some of the organs might be obtained. The liver, spleen, kidney, and suprarenals were minced up in both cases with the rabbit's own sera (which showed no immune body) and with saline. These sera and saline extracts were tested with ox blood corpuscles, to the saline extract being added as complement rabbit serum,—of itself non-lytic. No lysis was obtained with any of the organ extracts after incubating for two hours at 37° C.

Experiment 3.

It was thought that the rabbits in the last experiment had not been allowed to live long enough after the injection. A rabbit whose serum was ascertained by testing to be free from normal lysin for ox corpuscles was

injected intravenously with 2 c.c. ox blood corpuscles. Three days after injection it was killed by bleeding, and its serum was found to contain immune body. Nevertheless its serum was used to extract the organs of a fresh control rabbit, and its organs were extracted by the serum of this fresh control rabbit. These extracts were tested for immune body by addition of a suspension of ox blood corpuscle and incubation. No lysis took place, although the serum of the injected rabbit was of itself lytic to ox corpuscles.

Experiments 4 and 5.

Two rabbits without normal lysin for ox blood corpuscles in their blood were injected intravenously with ox blood corpuscles. Two days after this they were injected, one intravenously and one subcutaneously, with ox blood corpuscles to try to stimulate in the organ of formation, by the presence of these blood corpuscles, the production of immune body. Three hours after injection they were both killed by bleeding. Their serum showed no immune body, nor did extracts with the serum of the liver, kidney, spleen, or suprarenal.

Experiments 6 and 7.

Two rabbits were taken which had been injected frequently with ox blood corpuscles. The minimal hæmolytic dose of their serum, as regards immune body, when fully complemented, for 1 c.c. 1 per cent. ox blood corpuscles was about 0·002 c.c. They were killed by bleeding, and their organs—liver, spleen, kidney, and suprarenals—extracted with their own sera to see if any of the organ extracts contained more immune body than the corresponding serum. It was found that the minimal hæmolytic dose of the serum extracts as regards immune body was the same as the extracting serum, showing that there was no accession of immune body from any of the organs, and that the organ extracts did not absorb any of the immune body. But such quantities of these serum extracts as 1 c.c., owing to the absorption of the complement by the organ débris, gave no hæmolysis with a suspension of ox corpuscles, and this in spite of the strength of the sera recorded above.

The organ extracts were made by chopping up the tissues with a razor, adding the serum or saline and allowing to stand overnight at 8° C. They were then centrifuged and filtered through filter paper. In some cases they were even filtered through a Berkefeld filter. But this process was laborious, and where it succeeded it gave the same result.

I thus obtained no clue by the use of this method as to the seat of origin of immune body. I next tried to obtain some light on the subject by modified perfusion and by perfusion experiments on the liver.

Experiment 1.

A rabbit, which was ascertained by testing to have no normal lysin for ox blood corpuscles in its blood, was injected intravenously with 2 c.c. ox blood corpuscles. Two days after injection it was killed by bleeding from the carotid. As yet no immune body had appeared in its blood. The portal vein and inferior vena cava below the liver were ligatured, and a cannula was placed in the inferior vena cava above the liver. The liver was put in a saline bath at 37° C. It was then injected at a low pressure by means of the cannula in the inferior vena cava with 40 c.c. of 1 per cent. ox blood corpuscles in the rabbit's own serum. The liver was kept at 37° C. for two hours, and then some of the fluid withdrawn from the inferior vena cava. This fluid was found to be markedly hæmoglobin-tinted; but a control normal rabbit done at the same time gave the same result.

Experiment 2.

Experiment 1 was repeated and the same result obtained.

Experiment 3.

A rabbit which had been ascertained to have no normal lysin in its serum for ox blood corpuscles was injected intravenously with 2 c.c. ox blood corpuscles. Two days after this injection it was killed by bleeding. There was no immune body in its blood. The liver was excised, put in a saline bath at 37° C., and perfused with 1 per cent. ox blood corpuscles in saline. The corpuscular suspension was kept circulating with a Higginson's syringe at a low pressure, as judged by a mercury manometer. It was hoped that by this means the ox blood corpuscles might become sensitised with immune body. Samples were drawn off at various intervals, complement was added to them, and they were incubated. No hæmolysis occurred. No immune body appeared to have been given off to them.

Experiment 4.

Experiment 3 was repeated, with the same result.

From these experiments I obtained no positive information, nor did I obtain any from the following experiment.

Experiment 1.

A rabbit which had been immunised with ox blood corpuscles, and whose serum was very actively lytic to ox blood corpuscles, was injected intravenously with 4 c.c. ox blood corpuscles. Half an hour after injection it was killed by pithing. The abdomen and thorax were immediately opened and the liver ligatured out of the circulation. The animal was kept at room temperature for half an hour. Blood was then obtained from the liver and from the general circulation, and the minimal hæmolytic dose of immune body was ascertained in each case. It was found to be the same, and thus there had been no removal from, or accession to, the amount of immune body in the serum by its remaining in the liver.

Experiment 2.

Experiment 1 was repeated, with the same result. These direct experiments on the liver have failed, therefore, to give any positive information as to its being the seat of formation of immune body.

Conclusions.

1. Washed hen corpuscles, when injected intravenously into a rabbit whose serum lyses hen corpuscles *in vitro*, are very rapidly dissolved in the circulating plasma without any intervention of phagocytosis. This is some evidence for the view that the complement is free in the circulating plasma.

2. Further, when they are so injected, more especially when the rabbit's serum is not lytic *in vitro*, they tend very rapidly to accumulate in the liver, disappearing meanwhile from the blood and such organs as the spleen.

3. This accumulation in the liver, taken in conjunction with the known phagocytic power of the Kupffer cells, the metabolic activity of the liver cells, and their known action on the products of digestion, gives colour to the view that the liver may be the seat of formation of immune body.

4. The attempt to get information as to whether or not the liver is such a seat, by making extracts of the liver, fails because of the absorptive power of organ extracts for complement.

5. Various experiments noted above were done on the liver of rabbits to see if any facts pointing to it as a source of immune body could be obtained, but without success.

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SOME INVESTIGATIONS INTO THE PROBLEM OF THE ORIGIN OF IMMUNE BODY.[1]

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The literature on this subject is very confusing, owing to the lax use of such terms as "cytase" and "alexine." In certain papers one is led to believe that these are used as synonymous with the combination of complement and immune body. To a great extent this is due to the work having been done and the papers written, before Ehrlich differentiated the two substances in immune sera—the complement and immune body. But even after Ehrlich's work this confusion still continued, more especially with regard to the word cytase. I shall not, however, enter more fully here into this question than to say that in a question involving two variants—complement and immune body — one (the immune body) has tacitly been assumed to remain constant, and the variations of the other, the complement, have been observed under different conditions. In addition to this initial fallacy, observations which could only have a bearing on the origin of complement have, very illogically as I think, been applied to locate the origin of immune body.

Another fallacy has been introduced into the work on this subject. Some investigators have used extracts of various organs in different conditions to determine from what situation immune body was derived. But as von Dungern (1900 [7]), Muir (1903 [17]), and others have shown, tissue débris absorbs and renders inert large quantities of complement. A factor is thus introduced which cannot be controlled.

Bulloch (1901 [1]) was among the first to investigate the seat of origin of an immune body as such. He thought that if the leucocyte produced the immune body, a rise in their count in the blood stream ought to be observed during the process of immunising a rabbit with ox blood corpuscles. From results

[1] This work was done on a grant from the Carnegie Trustees, to whom I wish here to express my great indebtedness. [Received for publication April 15, 1910.]

obtained in this way he came to the conclusion that the immune body was formed from the mononuclears of the blood. Holmes (1905 [1]) observed the changes in the leucocytes of the blood of rabbits injected with foreign blood corpuscles. Though he makes no statement as to special leucocytes being the seat of formation of immune body, he concludes that the injection of these blood corpuscles produced a reaction exclusively affecting the mononuclear leucocytes.

Blood Count Observations on Rabbits injected with Ox Blood.

My attention was drawn to these papers, and arrested by the fact that both observers so clearly attribute a definite specific reaction to a definite set of blood cells, and I thought the matter worth inquiring into. Before, however, repeating their experiments I considered it a necessary preliminary to study the variations of the leucocytes as regards absolute and relative numbers in the normal rabbit. Brinkerhoff and Tyzzer (1902 [3]) give the following figures, for rabbits' leucocytes: Amphophils, 40 to 50 per cent.; eosinophils, 0·5 to 1 per cent.; mast cells, 4 to 8 per cent.; lymphocytes, 45 to 55 per cent.; large mononuclears, 2 to 8 per cent. As regards the total count in normal rabbits, they state that the leucocytes may vary in different individuals from 4600 per c.mm. to 13,400 per c.mm. Holmes [1] agrees with this latter statement. Lisin (1908 [4]) gives the following figures for leucocyte counts in the normal rabbit. The total leucocyte count in different individual normal rabbits may vary between 6000 and 14,000. For the differential count he gives the following figures: Neutrophil polymorphs, 32 to 45 per cent.; eosinophils, 0·5 to 4 per cent.; mast cells, 2 to 12 per cent.: lymphocytes and small mononuclears, 37 to 60 per cent.; large mononuclears, 2 to 5 per cent. I would particularly draw attention to the wide limits within which these figures may vary. In the first place, then, I examined the point as to whether variation in the ordinary laboratory feeding, time of day, etc., had of themselves any effect on the absolute or relative count of the leucocytes in the rabbit.

In determining this, the leucocytes were counted by means of the Zappert modification of the Thoma-Zeiss apparatus. Two different dilution samples were counted at each estimation. In counting, large squares were counted; and the number of leucocytes per c.mm. obtained by multiplying the average for each large square by 200. The figures given in the tables below are the average from the two samples. The differential counts were made by taking a drop of blood on one slide, spreading it uniformly by means of the edge of another slide, drying and staining with Leishman's stain. To obtain a good average of the whole slide 400 cells were counted, 100 being

[1] *Loc. cit.*

Experiment I.—Rabbit (buck).

Rabbit 1.

Date.	Hour.	Weight in Grms.	Gain in Weight.	Rectal Temperature.	Cabbage.[1]	Corn and Bran.[1]	Leucocytes.	Polymorphs.	Mononuclears.	Remarks.
					Since last observation			Per cent.	Per cent.	
June 18	9.20 a.m.	2300	..	39°	..	..	8,200	40	60	Fed on corn, bran, and cabbage at 10 o'clock.
,, 19	9.20 ,,	2370	+70	39°·2	..	..	7,400	26	74	..
,, 20	1.40 p.m.	2460	+90	39°·1	..	..	8,800	..	..	..
,, 20	11 ,,	2415	−45	..	..	..	..	..	..	..
,, 21	10 a.m.	2367	−48	..	..	..	..	..	..	Before being fed after this given at 10.30, 360 grms. cabbage and weighed
,, 21	2 p.m.	2455	+88	..	..	..	..	17	83	quantity corn and bran.
,, 21	6.30 ,,	2510	+55	39°·7	..	..	7,200	20	80	Had eaten 290 grms. cabbage
,, 21	10 ,,	2430	−80	..	..	..	..	..	..	and 97 grms.
,, 22	9.30 a.m.	2385	−45	..	..	..	..	..	..	corn and bran
					grms.	grms.				in twenty-four
,, 22	2.30 p.m.	2435	+50	39°·7	103	43	8,200	31	69	hours.
,, 22	6 ,,	2445	+10	..	62	7	..	..	..	..
,, 22	10 ,,	2450	+ 5	..	75	53	..	..	..	..
,, 23	9.40 a.m.	2435	−15	39°·5	60	32	10,000	17	83	..
,, 23	2 p.m.	2450	+15	..	53	30	..	..	..	..
,, 23	10 ,,	2530	+80	..	22	70	..	..	..	..
,, 24	9 a.m.	2460	−70	..	33	20	..	..	..	..
,, 24	2 p.m.	2510	+50	..	103	17	..	..	..	..
,, 24	6 ,,	2505	− 5	40°	64	19	7,600	28	72	..
,, 24	11 ,,	2487	−18	..	76	12	..	..	..	..
,, 25	9 a.m.	2420	−67	39°·5	55	22	7,600	18	82	..
,, 25	2 p.m.	2470	+50	39°·7	107	13	8,800	23	77	..
,, 25	10 ,,	2490	+20	..	135	50	..	..	..	..
,, 26	9 a.m.	2445	−45	39°·9	58	33	9,000	34	66	..
,, 26	2 p.m.	2470	+25	40°	88	25	7,000	21	79	..
,, 26	7 ,,	2470	+40	39°	78	26	6,200	28	72	..
,, 26	10 ,,	2470	−40	..	31	14	..	..	..	..
,, 27	9.45 a.m.	2457	−13	40°	103	38	9,000	13	87	..
,, 27	2 p.m.	2495	+38	40°	75	22	7,000	18	82	..
,, 27	6 ,,	2520	+25	39°·9	73	25	6,800	24	76	..
,, 27	10 ,,	2497	−23	..	38	23	..	..	..	..
,, 28	10 a.m.	2490	− 7	..	68	38	..	..	..	..
,, 29	10 ,,	2510	+20	39°·6	220	118	9,400	12	88	..
,, 29	2 p.m.	2530	+20	39°·8	43	20	10,600	23	77	All food removed from cages.
,, 29	6 ,,	2505	−25	39°·7	..	..	8,600	17	83	..
,, 29	10 ,,	2490	−15	..	..	..	..	..	..	..
,, 30	10 a.m.	2405	−85	39°·2	..	..	6,800	18	72	..
,, 30	2 p.m.	2500	+95	39°·8	95	22	7,600	23	77	All food removed from cages.
,, 30	6 ,,	2445	−55	39°·6	..	..	7,800	26	74	..
,, 30	10 ,,	2420	−25	..	..	..	..	..	..	..
July 1	10 a.m.	2390	−30	39°·5	..	..	7,400	14	86	..
,, 1	2 p.m.	2445	+55	39°·5	92	25	8,000	25	75	All food removed.
,, 1	6 ,,	2420	−25	39°·6	..	..	7,600	21	79	..
,, 1	10 ,,	2380	−40	..	..	..	..	..	..	..
,, 2	10 a.m.	2380	0	39°·8	..	..	6,800	17	83	..
,, 2	2 p.m.	2430	+50	39°·5	98	23	7,000	24	76	All food removed from cage.
,, 2	6 ,,	2415	−15	39°·6	..	..	6,200	25	75	..
,, 2	10 ,,	2400	−15	..	..	..	..	..	..	..
,, 3	10 a.m.	2350	−50	39°·5	..	..	7,400	22	78	..
,, 5	..	Three days interval to rest rabbits					..	..	.	Given cabbage, corn, and bran excess, food in the cages at the time.
,, 6	10 ,,	2300	.	39°·6	..		10,200	22	78	..
,, 6	2 p.m.	..		..	..	.	7,800	17	83	..
,, 7	10 a.m.	2350		39°·6	..	..	10,000	17	83	..
,, 7	3 p.m.			..	..	..	..	.		The rabbit was now injected subcutaneously with ox blood corpuscles. The results will be given later.

[1] These columns give the amount of food eaten since the last observation.

Experiment II.—Rabbit (buck).

Rabbit 2.

Date.	Hour.	Weight in Grms.	Gain in Weight.	Rectal Temperature.	Cabbage.[1]	Corn and Bran.[1]	Leucocytes.	Polymorphs.	Mononuclears.	Remarks.
								Per cent.	Per cent.	
June 18	10 a.m.	.	..	..	..	..	.	28	72	Fed at 10 o'clock on corn, bran, and cabbage.
,, 18	8 p.m.	..	..	..	..	..	..	23	77	..
,, 19	10 a.m.	1510	..	39°·2	..	..	12,400	24	76	..
,, 20	2 p.m.	1580	+70	39°·2	..	..	14,400	11	89	..
,, 20	11 ,,	1550	−30	..	.	..	..	..	..	..
,, 21	10 a.m.	1515	−35	..	..	.	..	..	..	..
,, 21	2 p.m.	1595	+80	..	..	..	..	..	..	..
,, 21	6 ,,	1620	+25	40° O.	..	..	10,600	16	84	..
,, 21	10 ,,	1620	0	..	..	..	..	..	.	..
,, 22	9 a.m.	1560	−60	..	grms. 290	grms. 45	..	..	..	Had eaten 290 grms. cabbage and 45 grms. corn and bran in twenty-four hours.
,, 22	3 p.m.	1570	+10	39°·9	68	38	12,600	19	81	..
,, 24	7 ,,	1580	+10	..	73	14	..	..	..	..
,, 22	10 ,,	1575	− 5	..	79	33	..	..	..	..
,, 23	10 a.m.	1570	− 5	39°·2	80	40	10,600	14	86	..
,, 23	2 p.m.	1570	0	..	70	..	..	..	..	..
,, 23	10 ,,	1615	+45	..	190	40	..	..	.	..
,, 24	10 a.m.	1555	−60	.	40	15	..	..	..	..
,, 24	2 p.m.	1620	+65	..	78	17	..	..	..	.
,, 24	7 ,,	1612	− 8	39°·9	78	13	13,800	15	85	.
,, 24	11 ,,	1630	+18	..	95	18	..	..	..	
,, 25	10 a.m.	1580	−50	39°·2	49	14	14,000	14	86	
,, 25	2 p.m.	1635	+55	39°·5	73	14	14,600	16	84	
,, 25	11 ,,	1675	+40	..	152	36	..	..	..	
,, 26	10 a.m.	1620	−55	39°·2	75	15	13,400	..	.	
,, 26	2 p.m.	1645	+25	39°·5	75	12	14,800	..	..	..
,, 26	8 ,,	1650	+ 5	39°·9	95	15	13,000	.	..	
,, 26	11 ,,	1607	−43	..	40	..	..	..	..	
,, 27	10 a.m.	1597	−10	39°·5	90	5	12,800	21	79	
,, 27	2 p.m.	1610	+13	39°·5	70	3	13,800	32	68	
,, 27	7 ,,	1605	− 5	39°·8	78	12	15,200	31	69	
,, 27	10 ,,	1620	+15	..	89	15	..	..	.	
,, 28	10 a.m.	1580	−40	..	63	12	..	..	..	..
,, 29	10 ,,	1617	+37	39°·2	258	60	13,400	20	80	..
,, 29	2 p.m.	1655	+38	39°·7	63	15	14,200	16	84	All food removed from cage.
,, 29	6 ,,	1620	−35	39°·5	..	..	12,200	18	82	..
,, 29	10 ,,	1590	−30	..	..	..	..	..	..	..
,, 30	10 a.m.	1565	−25	39°·2	..	..	10,000	14	86	..
,, 30	2 p.m.	1625	+60	39°·2	75	18	13,000	16	84	All food removed from cage.
,, 30	6 ,,	1575	−50	39°·5	..	..	12,400	15	85	..
,, 30	11 ,,	1535	−40	..	..	..	..	..	..	..
July 1	10 a.m.	1520	−15	39°	..	..	10,600	15	85	..
,, 1	2 p.m.	1620	+100	39°·5	135	12	14,200	..	..	All food removed from cage.
,, 1	6 ,,	1540	−80	39°·2	..	..	11,800	..	..	..
,, 1	11 ,,	1530	−10	..	..	..	..	..	..	..
,, 2	10 a.m.	1530	0	39°·2	..	..	12,400	19	81	..
,, 2	2 p.m.	1600	+70	39°·7	103	15	14,400	11	89	All food removed from cage.
,, 2	6 ,,	1530	+70	39°·5	..	..	12,600	13	87	..
,, 2	11 ,,	1498	−32	..	..	..	..	..	..	..
,, 3	10 a.m.	1485	−13	39°·2	..	..	12,600	10	90	..
,, 5	..	..	Three days interval				..	..	.	Fed on corn, bran, and cabbage to excess.
,, 6	10 a.m.	1515	..	39°·7	..	..	14,600	11	89	..
,, 6	2 p.m.	..	..	40°·2	..	..	13,600	21	79	..
,, 8	..	..	..	..	..	..	..	.	..	Killed, and found to be quite healthy in all its organs.

[1] These columns give the amount of food eaten since last observation.

Experiment III.—Rabbit (buck).

Rabbit 3.[1]

Date.	Hour.	Weight in Grms.	Gain in Weight.	Rectal Temperature.	Cabbage.[2]	Corn and Bran.[2]	Leucocytes.	Polymorphs.	Mononuclears.	Remarks.
								Per cent.	Per cent.	
June 19	10 a.m.	2020	..	40° C.	..	..	9,200	33	67	Fed at 10 o'clock on corn, bran, and cabbage.
,, 20	2 p.m.	2060	..	39°·1	..	..	8,800	42	58	
,, 20	11 ,,	2050	−10	..	..	..	..	..	..	..
,, 21	10 a.m.	2030	−20	..	..	..	..	..	..	..
,, 21	2 p.m.	2067	+47	..	..	..	..	..	..	..
,, 21	7 ,,	2050	−17	39°·8	..	.	6,400	47	53	..
,, 21	10 ,,	2047	− 3	..	..	..	..	..	..	..
,, 22	10 a.m.	2030	−13	..	grms. 290	grms. 20	..	..	..	Had eaten 290 grms. cabbage, and 20 grms. corn and bran in twenty-four hours.
,, 22	2 p.m.	2030	0	39°·9	68	13	12,600	34	66	
,, 22	7 ,,	2015	−15	..	56	3	..	..	..	
,, 22	10 ,,	2010	− 5	..	11	26	..	..	..	
,, 23	10 a.m.	2020	+10	40°·2	130	49	6,400	40	60	
,, 23	2 p.m.	2045	+25	..	65	..	..	..	..	..
,, 23	10 ,,	2020	−25	..	128	20	..	..	..	..
,, 24	10 a.m.	2005	−15	..	107	35	..	..	..	..
,, 24	2 p.m.	2050	+45	.	108	3	..	..	..	..
,, 24	7 ,,	2037	−13	39°·9	77	7	8,200	44	56	.
,, 24	11 ,,	2032	− 5	..	42	50	..	..	..	..
,, 25	10 a.m.	2015	−17	39°·9	54	27	8,200	34	66	..
,, 25	2 p.m.	2050	+35	39°·7	76	2	8,400	33	67	..
,, 25	10 ,,	2050	0	..	109	25	..	..	..	..
,, 26	10 a.m.	2050	0	40°·1	115	30	7,600	30	70	..
,, 26	2 p.m.	2070	+20	39°·9	48	..	8,800	32	68	..
,, 26	7 ,,	2030	−40	40° C.	52	..	8,400	47	53	..
,, 26	10 ,,	2040	+10	..	18	8	..	..	..	..
,, 27	10 a.m.	2035	− 5	39°·9	80	30	7,600	42	58	..
,, 27	2 p.m.	2020	−15	39°·9	63	..	7,200	50	50	..
,, 27	6 ,,	2005	−15	40	2	..	6,800	50	50	..
,, 27	10 ,,	1990	−15	..	5	7	..	..	..	..
,, 28	10 a.m.	1955	−35	..	21	21	..	..	..	..
,, 29	10 ,,	1955	0	..	115	43	6,400	35	65	..
,, 29	2 p.m.	1945	−10	39°·5	..	2	6,200	36	64	All food removed from cage.
,, 29	6 ,,	1935	−10	39°·5	..	..	6,400	50	50	..
,, 29	10 ,,	1925	−10	..	..	..	..	..	..	..
,, 30	10 a.m.	1900	−25	39°·2	..	..	6,600	34	66	..
,, 30	2 p.m.	1905	+ 5	39°·5	5	7	7,200	38	62	All food removed from cage.
,, 30	6 ,,	1890	−15	39°·5	..	..	5,400	32	68	..
,, 30	11 ,,	1885	− 5	..	..	..	..	..	..	..
July 1	10 a.m.	1860	−25	39°	..	..	10,600	38	62	..
,, 1	2 p.m.	1865	+ 5	39°·5	15	13	6,000	51	49	All food removed from cage.
,, 1	6 ,,	1855	−10	39°·5	..	..	6,200	30	70	..
,, 1	11 ,,	1845	−10	..	..	..	..	..	..	..
,, 2	10 a.m	1845	0	39°·5	..	..	4,800	30	70	..
,, 2	2 p.m.	1850	+ 5	39°·5	25	7	6,400	34	66	..
,, 2	7 ,,	1840	−10	39°·2	..	..	5,800	31	69	.
,, 2	11 ,,	1830	−10	39°·2	..	..	..	..	..	..
,, 3	10 a.m.	1810	−20	39°·2	..	..	5,400	31	69	
,, 5	..	..	..	Three days interval			..	..	..	Fed on corn, bran, and cabbage to excess.
,, 6	10 a.m.	1810	..	40	..	..	5,800	24	76	..
,, 6	2 p.m.	..	..	40	..	..	10,200	45	55	..

[1] Quite healthy, very excitable rabbit.

[2] These columns give the amount of food eaten since last observation.

taken from each of the two ends and each of the two sides of the film respectively. The leucocytes were counted simply as polymorphs and mononuclears.

The tame rabbit is a very irregular and capricious feeder, and its intestinal capacity is very great. A rabbit's stomach may be found half filled with food material forty-eight hours after its last meal. The liver cells twenty-four hours after a feed may show marked glycogenic changes, showing that active assimilation is going on even then. Joseph (1909 [13]) has shown that in rabbits taken at random from a laboratory stock the weight on the average of the stomach and cæcal contents is about 10 per cent. of the body weight. Taking these observations into consideration, I found that it would be impossible to estimate the leucocytes in a *fed* and in a *fasting* rabbit without introducing factors which would complicate or negative the experiment. Therefore, as a control basis, I endeavoured to find out what influence, if any, on the leucocyte count exaggerated laboratory feeding conditions would have. The experiments given in the tables above were made for this purpose. In the first place, the rabbits were given a limited amount of corn, bran, and cabbage, freedom being allowed them to eat at any time; next, they were allowed to eat as much as they could between 11 a.m. and 1 p.m., food being withheld for the rest of the day; and, lastly, they were given an excess of food with freedom to eat it when they chose.

From the tables it will be seen that the greatest variation in any of the rabbits between a minimum and a maximum estimation of the leucocytes is less than 5000. It will be seen from the tables that this and the lesser variations are not related to any concurrent change in the feeding, nor have they any relation to any particular time of day. With a limited number of observations, such as are possible in an experiment of this kind and with an apparatus such as the Thoma-Zeiss, I do not think that one can avoid an error of 3000 per c.mm. on either side of a mean. So that if a rabbit actually had 9000 leucocytes per c.mm., it might have equally well 12,000 or 6000 (unless the estimations of the leucocytes were very numerous), when estimated by means of the Thoma-Zeiss apparatus. So that I do not think that any variations in the leucocyte count noted above are at all attributable to variations in feeding or time of day.

With regard to the differential counts, a marked variation is shown by the figures given above, and this variation again has no relation to feeding or time of day. The variations, it will be seen, are quite erratic, and one is led to conclude that it is extremely difficult to draw any conclusions from the differential count in experimental conditions owing to the great variations possible under normal conditions. For instance, in rabbit 2 above, on June 16, in the differential count estimated from five slides made at the same time (to

test the accuracy of the method for estimating the differential count given above)—

The 1st slide showed	46	per cent.	polymorphs,	54	per cent.	mononuclears,
2nd	,,	42	,,	,,	58	,,	,,
3rd	,,	40	,,	,,	50	,,	,,
4th	,,	41	,,	,,	59	,,	,,
5th	,,	42	,,	,,	58	,,	,,

whilst on June 17 the differential count was 24 per cent. polymorphs, 76 per cent. mononuclears; and on June 18 it was 28 per cent. polymorphs, 72 per cent. mononuclears.

Again, in rabbit 10 (*infra*) the differential count on April 30 was 51 per cent. polymorphs and 49 per cent. mononuclears, while on May 5 it was 37 per cent. polymorphs and 63 per cent. mononuclears. This rabbit had had nothing done to it in the interval.

The conclusions to be drawn from these experiments then are, with regard to the effect of feeding and time of day on the absolute leucocyte count of the rabbit, that these have no noticeable effect; incidentally, with regard to the differential count in rabbits, that it is impossible to draw any conclusions from this in experimental conditions owing to the wide variation in normal conditions. Further, with regard to the figures obtained in an absolute count, to interpret them rightly, if a rabbit had actually 9000 leucocytes per c.mm., it ought to have at least 15,000 in an actual count (to cover errors of counting and normal variations in the animal) before it could be said to have a leucocytosis, and many more before far-reaching deductions could be drawn from it.

What effect, then, on the leucocyte count of rabbits has the injection (1) intravenously, (2) intraperitoneally, (3) subcutaneously, of washed ox blood? Experiments to determine this point were performed, and the results are given in tabular form below (pp. 269–275).

These experiments show conclusively that the injection into rabbits intravenously, intraperitoneally, or subcutaneously, of washed ox blood corpuscles does not produce a leucocytosis. The variations in the leucocyte count are not numerically of such a degree, nor do they appear at such a time after inoculation, to justify any relationship being set up between them and the appearance of the immune body. With regard to the differential count, for the reasons mentioned above one can draw no conclusions. If one were permitted to do so, from the figures given above, the conclusion would certainly not be that the mononuclears were relatively increased. I therefore submit that Bulloch had not sufficient basis for his conclusions. Whether any conclusions could be obtained by the method is another question. It is certainly one way of tackling the problem of the seat of origin of the immune body, but in my hands it has yielded negative

evidence, of a limited kind, only indicating that there is no stimulation of the leucocyte-forming organs. Such evidence is only of value when taken in conjunction with results obtained from many other methods of attacking the question.

TABLES SHOWING EFFECT ON THE LEUCOCYTE COUNT OF RABBITS OF INGESTING WASHED OX BLOOD CORPUSCLES INTRAVENOUSLY, INTRAPERITONEALLY, AND SUBCUTANEOUSLY.

I. INTRAVENOUSLY.

Experiment I.—Rabbit (buck).

RABBIT 4.—BLACK, CROPPED FOREHEAD.

Date.	Hour.	Leucocytes.	Remarks.	Date.	Hour.	Leucocytes.	Remarks.
Feb. 7	2 p.m.	11,200	...	Feb. 20	10 a.m.	11,500	...
,, 8	10 a.m.	15,700	...	,, 21	2 p.m.	10,200	3 c.c. ox blood corpuscles + 2 c.c. saline intravenously at 2.30 on 20th
,, 9	2 p.m.	11,800	...				
,, 10	7 ,,	10,200	...				
,, 11	7 ,,	8,700	...				
,, 12	10 a.m.	9,900	...				
,, 13	1 p.m.	11,900	...	,, 22	2 ,,	9,200	
,, 14	10 a.m.	12,700	...	,, 23	2 ,,	8,000	...
,, 15	7 p.m.	10,800	...	,, 24	2 ,,	9,300	Immune body present
,, 16	2 ,,	10,600	...	,, 25	2 ,,	10,800	
,, 17	10 a.m.	10,700	...	,, 29	9 a.m.	8,800	...
,, 18	7 p.m.	8,900	...	,, 30	2 p.m.	10,800	...

Experiment II.—Rabbit (buck).

RABBIT 5.—BLUE AND WHITE.

Date.	Hour.	Leucocytes.	Remarks.	Date.	Hour.	Leucocytes.	Remarks.
Feb. 7	2 p.m.	8,400	...	Feb. 20	10 a.m.	11,300	3 c.c. ox blood corpuscles + 2 c.c. saline intravenously at 2.30 on 20th
,, 8	9 a.m.	16,900	...				
,, 9	2 p.m.	9,800	...				
,, 10	7 ,,	8,400	...	,, 21	10 ,,	7,800	
,, 11	7 ,,	10,200	...	,, 21	7 p.m.	10,300	
,, 12	10 a.m.	12,000	...	,, 22	10 a.m.	8,800	
,, 13	2 p.m.	12,200	...	,, 23	10 ,,	8,800	Immune body present
,, 14	9 a.m.	11,600	...	,, 23	7 p.m.	10,000	
,, 15	6 p.m.	8,600	...	,, 24	10 a.m.	9,400	...
,, 16	2 ,,	10,700	...	,, 25	10 ,,	8,500	...
,, 17	10 a.m.	12,600	...	,, 26	10 ,,	8,500	...
,, 18	7 p.m.	9,400	...	,, 27	10 ,,	9,500	...
				,, 28	10 ,,	11,300	...
				,, 30	10 ,,	9,900	...

Experiment III.—Rabbit (buck).

Rabbit 6—White, marked Green on Forehead.

Date.	Hour.	Leucocytes.	Remarks.	Date.	Hour.	Leucocytes.	Remarks.
Feb. 8	10 a.m.	11,800	...	Feb. 20	7 p.m.	10,000	...
,, 9	3 p.m.	8,000	...	,, 21	7 ,,	9,000	...
,, 10	8 ,,	9,000	...	,, 22	10 a.m.	8,400	...
,, 11	8 ,,	9,700	...	,, 22	6 p.m.	8,600	...
,, 12	10 a.m.	9,700	...	,, 23	10 a.m.	9,300	...
,, 13	2 p.m.	10,000	...	,, 23	6 p.m.	8,900	Immune body in blood.
,, 14	10 a.m.	8,800	...				
,, 15	7 p.m.	8,700	...	,, 24	10 a.m.	9,800	...
,, 16	3 ,,	9,300	...	,, 24	7 p.m.	9,600	...
,, 17	10 a.m.	11,100	...	,, 25	6 ,,	11,300	...
,, 18	7 p.m.	10,400	...	,, 26	10 a.m.	8,400	...
,, 20	10 a.m.	11,100	3 c.c. ox blood corpuscles + 2 c.c. saline intravenously at 2.30 on 20th	,, 27	2 p.m.	9,000	...
				,, 28	3 ,,	8,500	...
				,, 29	2 ,,	7,600	...
				,, 30	10 a.m.	10,200	...
				Mar. 2	2 p.m.	10,000	...

Experiment IV.—Rabbit (buck).

Rabbit 7—White, Cropped Rump.

Date.	Hour.	Leucocytes.	Remarks.	Date.	Hour.	Leucocytes.	Remarks.
Feb. 8	10 a.m.	13,800	...	Mar. 21	10 ,,	10,600	...
,, 9	2 p.m.	10,400	...	,, 22	10 a.m.	10,800	...
,, 10	7 ,,	8,200	...	,, 22	6 p.m.	8,600	...
,, 11	7 ,,	11,300	...	,, 23	10 a.m.	8,600	Immune body present.
,, 12	10 a.m.	15,400	...				
,, 13	2 p.m.	12,000	...	,, 24	10 ,,	10,800	...
,, 14	10 a.m.	11,700	...	,, 24	6 p.m.	8,100	...
,, 15	7 p.m.	9,900	...	,, 25	2 ,,	10,800	...
,, 16	2 ,,	10,500	...	,, 26	10 a.m.	10,000	...
,, 17	10 a.m.	13,900	...	,, 28	10 ,,	10,200	...
,, 18	7 p.m.	9,400	...	,, 30	2 p.m.	9,900	...
,, 20	10 a.m.	13,800	3 c.c. ox blood corpuscles + 2 c.c. saline intravenously at 2.30 on 20th				

Experiment V.—Rabbit (buck).[1]

RABBIT 8.

Date.	Hour.	Weight in Grms.	Rectal Temperature.	Leucocytes.	Polymorphs.	Mononuclears.	Remarks.
					Per cent.	Per cent.	
April 20	10 a.m.	2430	39°·2	7,300	69	31	...
,, 22	12 ,,	2430	39°·2	7,600	37	63	...
,, 23	12 ,,	...	...	9,400	...	...	Bled 3 c.c. from ear vein.
,, 23	1.30 p.m.	...	...	...	...	...	Injected intravenously 3 c.c. ox blood corpuseles + 2 c.c. saline.
,, 23	3 ,,	...	39°·9	9,600	50	49	
,, 24	2 ,,	2460	39°·2	10,000	38	62	
,, 25	11 a.m.	...	...	8,500	22	78	...
,, 27	10 ,,	...	39°·7	8,400	...	...	...
May 18	10 ,,	2960	...	7,500	...	...	...

Experiment VI.—Rabbit (buck).

RABBIT 9.

Date.	Hour.	Weight in Grms.	Change in Weight.	Leucocytes.	Polymorphs.	Mononuclears.	Rectal Temperature.	Remarks.
					Per cent.	Per cent.		
July 4	10 a.m.	1745	...	6,400	40	60	39°·2	...
,, 5	10 ,,	1715	...	7,800	18	82	39°·2	..
,, 5	4 p.m.	1705	− 10	6,200	20	80	39°·2	..
,, 6	10 a.m.	1660	− 45	8,000	18	82	39°·9	...
,, 6	3 p.m.	...	...	7,800	28	72	39°·9	...
,, 7	10 a.m.	1670	..	9,200	16	84	39°·2	...
,, 7	2 p.m.	1780	+110	10,800	28	72	39°·2	7 c.c. ox blood corpuscles + 3 c.c. saline intravenously at 3 p.m. of 7th.
,, 7	6 ,,	1800	+ 20	10,800	30	70	39°·1	
,, 8	10 a.m.	1780	− 20	9,400	29	71	39°·1	
,, 8	2 p.m.	1810	+ 30	7,800	23	77	39°·2	
,, 8	7 ,,	1860	+ 50	10,600	28	72	39°·5	..
,, 9	10 a.m.	1830	− 30	8,600	23	77	39°·1	...
,, 9	2 p.m.	1840	+ 10	9,400	25	75	39°·2	..
,, 9	6 ,,	1870	+ 30	9,200	20	80	39°·5	...
,, 10	10 a.m.	1850	− 20	8,200	19	81	39°·2	..
,, 10	2 p.m.	1845	− 5	8,400	35	65	39°·9	..
,, 10	7 ,,	1860	+ 15	9,000	30	70	39°·5	..
,, 11	10 a.m.	1850	− 10	7,400	20	80	39°·1	..
,, 12	10 ,,	1900	+ 50	10,700	18	82	39°·0	..
,, 12	2 p.m.	1900	0	8,000	17	83	39°·0	..
,, 12	6 ,,	1900	0	10,200	15	85	39°·0	..
,, 13	10 a.m.	1880	− 20	9,400	22	78	39°·2	...
,, 13	2 p.m.	1880	0	9,800	25	75	39°·0	...
,, 14	10 a.m.	1900	+ 20	10,400	27	73	39°·2	
,, 14	2 p.m.	1880	− 20	8,400	16	84	39°·1	...
,, 15	11 a.m.	1875	− 5	9,800	18	82	39°·1	...
,, 16	12 ,,	1940	+ 65	10,200	19	81	39°·2	..
,, 17	12 ,,	1885	− 55	9,200	20	80	39°·2	...

[1] In Experiments V.–IX. the date of the appearance of the immune body was not determined,—to exclude the possibility of the daily bleeding for this purpose having an effect on the leucocyte count.

Experiment VII.—Rabbit (buck).

Rabbit 10—Cage 28—Angora, Cropped Head.

Date.	Hour.	Weight in Grms.	Rectal Temperature	Leucocytes.	Polymorphs.	Mononuclears.	Remarks.
					Per cent.	Per cent.	
April 30	11 a.m.	2065	40°	8,000	51	49	...
May 5	11 ,,	2080	40°	8,700	37	63	...
,, 5	1 p.m.	...	...	...	...	...	Bled 4 c.c.
,, 5	1.10 ,,	...	...	...	...	...	Injected intravenously with 2 c.c. ox blood corpuscles washed + 2 c.c. saline.
,, 5	1.30 ,,	...	40°·1	9,200	47	53	
,, 6	10 a.m.	...	39°·5	8,400	...	...	...
,, 7	10 ,,	...	39°·9	8,800	...	...	...
,, 8	10 ,,	...	39°·8	8,200	...	...	...
,, 9	10 ,,	...	40°·5	8,600	...	...	...
June 4	10 ,,	2270	40°·1	10,600	40	60	...
,, 9	10 ,,	2285	40°·5	7,800	34	66	...

Experiment VIII.—Rabbit (white buck).

Rabbit 11—Cage 27.

Date.	Hour.	Weight in Grms.	Rectal Temperature.	Leucocytes.	Polymorphs.	Mononuclears.	Remarks.
					Per cent.	Per cent.	
May 4	2 p.m.	1788	39°·2	10,000	58	42	..
,, 6	10 a.m.	1755	39°·2	9,200	43	57	...
,, 6	1.15 p.m.	...	...	...	...	...	Bled 4 c.c.
,, 6	1.30 ,,	...	...	...	...	...	2 c.c. ox blood corpuscles + 2 c.c. saline intravenously.
,, 6	2.30 ,,	...	39°·5	9,900	75	25	
,, 7	10 a.m.	...	39°·8	8,600	...	...	...
,, 8	10 ,,	...	39°·8	8,400	...	...	...
,, 9	10 ,,	...	39°·5	9,400	...	...	...
June 4	10 ,,	1910	39°·2	7,400	...	...	...
,, 9	10 ,,	1880	39°·5	7,200	...	...	...

Experiment IX.—Rabbit (buck).

Rabbit 12—Black, Cropped Forehead—Cage 25.

Date.	Hour.	Weight in Grms.	Rectal Temperature.	Leucocytes.	Polymorphs.	Mononuclears.	Remarks.
					Per cent.	Per cent.	
April 27	2 p.m.	2670	39°·2	9,000	58	42	...
,, 28	2 ,,	...	39°·2	7,400	55	45	...
,, 28	3 ,,	...	...	7,200	51	49	...
May 1	10 a.m.	2675	39°·0	8,900	64	36	...
,, 1	1.30 p.m.	...	...	...	...	...	2 c.c. ox blood corpuscles +2 c.c. saline intravenously.
,, 1	2 ,,	...	39°·2	8,400	59	41	...
,, 2	10 a.m.	...	39°·5	...	59	42	...
,, 4	10 ,,	...	39°	...	51	49	...
,, 5	10 ,,	...	39°	8,000	56	44	...
,, 17	...	2730	39°·2	9,600	72	28	...

II. INTRAPERITONEALLY.

Experiment I.—Rabbit (buck).

Rabbit 13—Small Angora—Cage 31.

Date.	Hour.	Weight in Grms.	Rectal Temperature.	Leucocytes.	Polymorphs.	Mononuclears.	Remarks.
					Per cent.	Per cent.	
June 10	10 a.m.	1420	39°	11,200	24	76	...
,, 11	10 ,,	1410	39°	9,000	40	60	...
,, 12	10 ,,	1460	39°	8,800	38	62	2 c.c. ox blood corpuscles intraperitoneally at 10 a.m.
,, 12	11 ,,	...	...	...	42	58	
,, 13	10 ,,	...	39°	11,000	51	49	No local pain and no swelling.
,, 14	10 ,,	...	39°	8,200	33	67	,,
,, 15	10.30 ,,	1480	39°	7,600	31	69	,,
,, 16	10 ,,	...	39°	10,600	34	66	,,
,, 17	10 ,,	1440	39°	7,400	31	69	,,
,, 18	10 ,,	...	39°	10,000	31	69	,,
,, 19	10 ,,	...	38°·7	7,400	32	68	Immune body present in serum.
,, 20	10 ,,	1380	39°	7,400	55	45	...
,, 21	10 ,,	...	39°	7,800	33	67	...

A second rabbit was injected intraperitoneally, but it developed diarrhœa, so that it was not kept under observation further.

III. SUBCUTANEOUSLY.

Experiment I.—Rabbit (buck).

Rabbit 14—Cage 30.

Date.	Hour.	Weight in Grms.	Rectal Temperature.	Leucocytes.	Polymorphs.	Mononuclears.	Remarks.
					Per cent.	Per cent.	
May 11	10 a.m.	2130	39°·2	7800	35	65	...
,, 12	1 p.m.	2117	39°·1	...	...	...	2 c.c. ox blood corpuscles subcutaneously at 2.25.
,, 13	10 a.m.	...	39°	8200	31	69	
,, 15	10 ,,	...	39°·2	9600	...	...	No local swelling.
,, 16	10 ,,	...	39°·7	7200	...	...	,,
,, 17	2 p.m.	...	39°	6900	...	...	,,
,, 19	10 a.m.	...	39°	9200	...	...	,,
,, 21	10 ,,	2145	40°	9700	39	61	Immune body present.
,, 25	10 ,,	2140	39°	8600	...	...	...
June 4	10 ,,	2260	39°·5	8100	28	72	...
,, 9	10 ,,	2145	39°·2	9000	27	73	...

Experiment II.—Rabbit (buck).

Rabbit 15—Cage 29.

Date.	Hour.	Weight in Grms.	Rectal Temperature.	Leucocytes.	Polymorphs.	Mononuclears.	Remarks.
					Per cent.	Per cent.	
May 11	10 a.m.	2182	39°·5	7800	30	70	...
,, 12	10 ,,	2205	39°·2	6000	52	48	...
,, 12	12 ,,	...	...	...	...	...	Subcutaneously 2 c.c. ox blood corpuscles.
,, 13	11 ,,	...	39°·2	8100	51	49	No pain and no swelling.
,, 15	10 ,,	...	39°·2	8700	...	...	,, ,,
,, 16	10 ,,	...	39°·5	7600	...	...	,, ,,
,, 17	2 p.m.	...	40°·2	7100	...	...	,, ,,
,, 19	10 a.m.	...	39°·2	9100	...	...	,, ,,
,, 21	10 ,,	2215	39°·2	9000	...	...	Immune body present.
,, 25	10 ,,	2222	40°	8000	32	68	...
June 2	10 ,,	2185	...	...	...	...	...
,, 4	10 ,,	2235	40°·2	8600	34	66	...

Experiment III.

Rabbit used is rabbit 1, which was used previously for estimating the effect of food variation on the leucocytes. The rabbit was given, while these observations were being made, corn, bran, and cabbage in excess.

Date.	Hour.	Weight in Grms.	Rectal Temperature.	Leucocytes.	Polymorphs.	Mononuclears.	Remarks.
					Per cent.	Per cent.	
July 6	10 a.m.	2300	39°·6	10,200	22	78	...
,, 6	2 p.m.	..	...	7,800	17	83	...
,, 7	10 a.m.	2350	39°·6	10,200	17	83	...
,, 7	11 ,,	...	...	...	...	...	8 c.c. ox blood corpuscles injected subcutaneously 2 c.c. in four different places.
,, 7	2 p.m.	2470	39°·8	8,600	19	81	No swelling, and no pain.
,, 7	6 ,,	2510	39°·8	8,400	36	64	...
,, 8	10 a.m.	2510	39°·8	8,400	29	71	...
,, 8	2 p.m.	2510	39°·0	7,600	30	70	...
,, 8	7 ,,	2545	39° 3	9,200	15	85	...
,, 9	10 a.m.	2520	39°·8	10,000	10	90	...
,, 9	2 p.m.	2510	39°·3	7,800	16	84	...
,, 9	6 ,,	2550	39°·3	6,200	30	70	...
,, 10	10 a.m.	2540	39°·1	10,000	26	74	...
,, 10	2 p.m.	2540	39°·4	7,600	21	79	...
,, 10	7 ,,	2550	39°·1	8,400	20	80	...
,, 11	10 a.m.	2530	39°·2	8,800	14	84	...
,, 12	10 ,,	2580	39°·5	9,200	22	78	...
,, 12	3 p.m.	2570	39°·5	8,200	19	81	...
,, 12	6 ,,	2560	39°·1	8,000	26	74	...
,, 13	10 a.m.	2580	39°·1	9,800	13	87	...
,, 13	3 p.m.	2560	39°·0	8,200	23	77	...
,, 14	10 a.m.	2625	39°·2	10,200	18	82	...
,, 14	2 p.m.	2600	39°·2	9,200	10	90	...
,, 15	11 a.m.	2645	39°·0	9,200	12	88	...
,, 16	11 ,,	2635	38°·9	7,800	16	84	...
,, 17	12 ,,	2585	38°·9	8,200	14	86	...

Effect of Removal of Organs on Appearance of Immune Body in Blood.

The problem of determining the seat of origin of the immune body is a very difficult one, and the determination has to be attempted mostly by indirect methods. A result obtained from one of these indirect methods is of very little value by itself, and it is only when taken in conjunction with other results obtained by other indirect methods that any clue can be obtained.

A prominent rôle in the production of immune body has been attributed to the spleen by many investigators, including Metchnikoff. London (1901 [12]) splenectomised guinea-pigs and found that the

removal of the spleen prevented the formation of immune body to foreign blood corpuscles injected subcutaneously.

Tarassevitch (1902 [24]), Jakuschawitsch (1906 [12]), on the other hand, found that the removal of the spleen had no effect on the production of hæmolytic immune body. Considering the divergent results obtained, I deemed it necessary to repeat these experiments with modifications. Using rabbits as the experimental animals, I determined the effect of removing the spleen as well as other bodily organs. In the case where the spleen was removed I also counted the leucocytes during the experiment to exclude a vicarious action on the part of the other leucocyte-forming organs. The principal modification I introduced was to use a time standard in estimating the production of the immune body. As Sachs (1903 [22]) and others showed, and I myself corroborated, rabbits when injected intravenously with ox blood corpuscles produce immune body critically on or about the third day after injection. The introduction of this time standard of production of immune body involved considerable difficulty, for rabbits had to be selected whose serum was not of itself naturally hæmolytic to ox blood corpuscles. The test applied for exclusion of rabbits with lytic serum was that their serum on three occasions at intervals of three days with three different samples of ox blood should show no trace of hæmolysis (the serum being added to 1 c.c. 1 per cent. suspension of the ox blood corpuscles and incubated for two hours at 37° C.). This involved the examination of a considerable number of rabbits to obtain a very small number of animals whose serum fulfilled the condition mentioned. This small number was further reduced by deaths after the operation.

Experiment 1.

Removal of the Spleen.

Rabbit (buck).—Selected as above with regard to absence of lysin normally in its blood serum and splenectomised. Its serum was tested again two weeks after splenectomy for lysin: none was present. It was then injected intravenously into the ear vein with 3 c.c. washed ox blood corpuscles + 2 c.c. saline. Thereafter the serum was examined daily by adding 1 c.c. of it to 1 c.c. 1 per cent. ox blood corpuscles, and incubating at 37° C. for two hours. Hæmolysis was obtained for the first time on the third day, showing the presence of immune body. The removal of the spleen thus had had no effect on the production of the immune body or on the time of its appearance.

Experiment 2.

Removal of the spleen and counting of the leucocytes.

Rabbit (buck).—Selected as above with regard to absence of lysis normally in its blood serum. Leucocytes found to vary between 9000 and 12,000 per c.mm. Spleen removed. Serum tested again three days after operation, and hæmolysin found to be absent. Leucocytes counted and found to be 10,000 per c.mm. Rabbit then injected intravenously into ear vein with 3 c.c. washed

ox blood corpuscles + 2 c.c. saline. Serum tested daily after injection as before for immune body and leucocytes counted. The immune body appeared for the first time on the third day and no leucocytosis was observed. This would appear to exclude a vicarious action on the part of the leucocyte-forming organs in the formation of immune body when the spleen is removed.

Experiment 3.

Thyroid removed.

Rabbit (buck, selected as above).—Thyroid removed. No hæmolysin present in blood serum two weeks after the operation. Rabbit then injected intravenously into the ear vein with 3 c.c. washed ox blood corpuscles + 2 c.c. saline. Blood serum tested daily as before for immune body. The immune body first appeared in the blood serum on the third day. The removal of the thyroid had, therefore, no effect on the time of production of the immune body.

Experiment 4.

Spleen and Thyroid removed.

Rabbit (buck, selected as above).—Thyroid removed seven days after spleen removed: on being tested, two weeks after this no hæmolysin was found in its blood serum. It was then injected intravenously into the ear vein with 3 c.c. washed ox blood corpuscles + 2 c.c. saline. The blood serum was tested daily after the injection for immune body, and its presence was detected for the first time on the third day. It would appear, therefore, that removal of the spleen and thyroid in the same animal caused no delay in the appearance of the immune body.

Experiment 5.

Removal of one Kidney.

Rabbit (buck).—Selected as before. One kidney removed. Hæmolysin was found to be absent from the blood serum two weeks after the operation. The rabbit was then injected intravenously with 3 c.c. washed ox blood corpuscles + 2 c.c. saline. The blood serum was tested daily after the injection for the appearance of immune body, which appeared for the first time on the third day. Removal of one kidney then had no influence on the time of appearance of the immune body.

To summarise the results of these experiments then, removal of the spleen, spleen and thyroid, thyroid, or one kidney from rabbits had no influence on the time of appearance of the immune body in the blood. Where the spleen was removed, the animal injected, and the leucocytes counted, no leucocytosis was observed, and thus no evidence, such as it is, for a vicarious action of other leucocyte-forming organs is gained.

Metchnikoff has used the results obtained from one other method of research—that of obtaining hæmolytic substances in extracts of what he terms macrophagic organs such as the spleen—to support his view of the origin of macrocytase. I shall only mention here again the difficulty in the technique introduced by the fact of the absorption of the complement by the tissue débris, and that while Metchnikoff

holds that his macrocytase is derived solely from what he terms macrophagic organs, others have shown that it can be derived equally well from many other organs, *e.g.* pancreas. Further, the substance itself, macrocytase, has nothing in common with the alexine or the compound of complement and immune body that one finds in the serum.

Production of Anti-bodies by Feeding.

The method by which the circulating fluids of the body normally come into contact with foreign albuminous matter is by way of the intestinal canal. The greater portion of this albuminous material is altered by digestion before it is absorbed, but there is the possibility that, as a more or less usual process, some minute portions of it may be absorbed unchanged. The formation of anti-bodies then would be part of a process that goes on to a small degree in so-called normal conditions. This normal process might be exaggerated when a large quantity of the foreign albumin obtained entrance by some other abnormal path, such as by injection subcutaneously, intraperitoneally, or intravenously. The formation of anti-bodies then would be a normal process of the body for which an apparatus, normally working at slow but capable of working at much higher rate, was already prepared; as the intestine is the normal supplier of this foreign albumin, etc., this apparatus one might naturally suppose would be in relation to it. The liver receives all the blood, with its contained material from the intestine, and it is well known that with respect to certain substances, *e.g.* glucose, profoundly modifies it. The liver also has been shown to neutralise in large quantities pharmacological substances and toxins (strictly so called).

Especially has this been shown to be the case by Italian observers, Petrone and Pagano (1907 [20]), with regard to toxins of intestinal origin. It is necessary to inquire, therefore, whether the liver may not be a possible seat of formation of immune body. I shall first give a brief résumé of the literature concerning the production of anti-bodies by feeding animals on the corresponding antigens. Ehrlich (1891 [8]) produced immunity in animals against ricin and abrin by feeding them on these substances. Fraser (1895 [10]) produced a similar effect by feeding animals on snake venom. Uhlenhuth (1900 [26]), Ascoli (1903 [1]), Cantacuzène (1907 [5]), Sternberg (1908 [23]), Petit and Jean Minet (1908 [19]), have produced specific precipitins in animals by feeding them on various sera. Metchnikoff (1901 [16]) fed rats on rabbit and horse blood, and produced specific haemolysins and agglutinins for rabbit and horse blood. Celler and Hamburger (1905 [6]), however, criticise these experiments by saying that Metchnikoff had not excluded the possibility of a natural lysin for rabbit and ox blood corpuscles being present in rat serum. Rosenau and Anderson (1908 [21]) sensitised guinea-pigs by feeding them on horse serum. Many writers, too, have brought forward evidence of the production of anti-bodies to pathogenic organisms by feeding animals on these organisms. Tchitchkine (1904 [25]) produced anti-bodies to typhoid bacilli in this way.

Breton and Petit (1909 [2]) produced antitoxin to diphtheria by giving

diphtheria cultures by the mouth, and Fornario (1908 [9]) vaccinated rats and rabbits against plague by feeding them on cultures of plague. With regard to these last experiments with living cultures the criticism is valid that some lesion may have been produced in the intestinal tract of the animals. With regard to the experiments with various sera and blood corpuscles, the animal in some cases was fed with a stomach tube, which might have produced some gastric lesion and absorption of the antigen through a broken surface. Again, in these experiments due care was not always taken to exclude the possibility of a normal anti-body being present in the animal's blood before commencing feeding the animal. I therefore thought it would be interesting to repeat the experiments, due regard being paid to these points.

In my experiments I used as the experimental animals rabbits, and as the antigen whole ox blood. As in the experiments already given, I excluded animals with a normal lysin for ox blood corpuscles. The presence of a normal agglutinin or precipitin was also excluded. Only when the animal had passed these different tests was it considered fit to be experimented on. The rabbits were then kept in separate cages and fed on ox blood, 2 oz. of fresh blood being stirred up thoroughly amongst their corn and bran each morning. The feeding dish was cleaned out thoroughly every morning. After a few days the rabbits began to eat this mixture quite well.

Experiment I.

Rabbit (brown doe).—Tested as above for presence of normal lysin, agglutinin, and precipitin for ox blood from 6th to 8th December. Feeding on blood commenced on 10th December. Animal's serum tested about every week thereafter for the appearence of anti-bodies. Immune body, agglutinin, and precipitin to ox blood first found on 15th January.

Experiment II.

Rabbit (buck).—Tested as above between 6th and 8th December. Feeding on blood commenced on 10th December. Animal's serum tested about every week thereafter for the appearance of anti-bodies. Immune body, agglutinin, and precipitin noticed first about 20th January.

Experiment III.

Rabbit (brown, rough).—Tested as above from 29th to 31st January. Feeding on ox blood commenced 31st January. On 15th February no anti-bodies were observed to be present. On 21st February immune body, agglutinin, and precipitin were noticed to be present.

Experiment IV.

Rabbit (brown rough, eosin mark).—Tested as above from 29th to 31st January. Feeding on ox blood commenced 31st January. On 15th February immune body, precipitin, and agglutinin to ox blood were found to be present in the serum.

Thus in these experiments, with carefully tested animals, I was able to produce a hæmolysin, an agglutinin, and a precipitin to ox blood by feeding rabbits on ox blood.

Conclusions.

1. The exclusion of such organs as the spleen, the leucocyte and lymphatic apparatus, the thyroid or the kidney, by extirpation and other methods, does not delay the critical production of the immune body on the third day after the rabbit has been injected intravenously with ox blood corpuscles. I consider this strong evidence that the immune body is not formed in these organs.

2. There is no leucocytosis observable in the rabbit after an intravenous, intraperitoneal, or subcutaneous injection of ox blood corpuscles. This further excludes the leucocytic organs (as far as they can be excluded by such a method) from a participation in the production of immune body.

3. When rabbits are fed on ox blood, a hæmolysin, an agglutinin, and a precipitin against ox blood is produced. One might therefore suspect that the production of anti-bodies is the exaggeration of a process which normally deals with foreign and unaltered proteid gaining admission to the circulation through the walls of the intestine. That it is produced in large quantity in ordinary immunising experiments may be accounted for by the larger quantity of undigested material that gains entrance to the circulation under such circumstances.

4. I have shown elsewhere (1910[18]) that the liver has a great power of sifting out particulate foreign material from the circulation. One hesitates to attribute to any single organ the work of producing anti-bodies; but taking this into consideration, along with the embryological derivation of the liver, its situation directly in the path of foreign material normally entering the circulation, the known importance of the liver in secondary digestion, and its great metabolic activity, more attention might be paid to the possibility of its playing a rôle in the formation of anti-bodies than has hitherto been done.

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NOTE ON THE OCCURRENCE OF A TRYPANOSOME (TRYPANOSOMA CUNICULI, BLANCHARD) IN THE RABBIT.[1]

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WITH A NOTE ON THE EXPERIMENTAL WORK

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The object of the present note is to record the occurrence of *Trypanosoma cuniculi*, Blanchard (1904[2]), which was observed by one of us while examining the blood of tame rabbits which had been bred either in or around Edinburgh. We also take the opportunity of adding a few observations on the morphology of the parasite, and on its effect upon the host.

This trypanosome was first observed by Jolyet and de Nabias of Bordeaux in July 1882, but was not recorded by them until 1891; they found the organism in four out of ten rabbits examined. Nocard and Leclainche (1903[7]) refer, in passing, to the presence of a trypanosome in rabbits. Petrie (1904[8]) first recorded this organism in British rabbits. While examining tame rabbits at Elstree, Hertfordshire, in May 1902, he accidentally discovered that the blood of one of them swarmed with trypanosomes; two other infected rabbits were observed in January 1903, but seventy other tame rabbits were examined with negative results. In a later paper (1905[9]) Petrie states that tame rabbits are seldom infected, for 230 were examined with negative results, but out of forty wild rabbits four proved to be infected. Bose (1906[3]) records the presence of the same trypanosome in a wild rabbit, one of a dozen bought in the market of Montpellier, and Bettencourt and Franca (1906[1]) found the organism in three out of eleven rabbits from Thomar (Estremadura), but in sixty-nine rabbits from other districts of Portugal no trypanosomes could be found. Trypanosomes similar to those described by Petrie were recorded by Manca (1906[6]) in the blood of a wild rabbit from Sardinia. Nicolle (see Laveran and Mesnil, "Trypanosomes et Trypanosomiases, 1904," pp. 100–101, English edition, 1907, p. 104) observed trypanosomes in the blood of rabbits in Constantinople, but no morphological details regarding this trypanosome are available. The above are, as far as we are aware, all the

[1] Received January 28, 1909.

definite first-hand records of the spontaneous occurrence of trypanosomes in the blood of rabbits.

We found *T. cuniculi* in the blood of three out of eighty-nine tame rabbits, obtained from Edinburgh and district, examined in May 1908. Although other rabbits from the same source, including a batch of forty-eight, in January 1909, have been examined from time to time, no further cases showing the presence of this organism have been met with.

The trypanosome of the rabbit was described by Jolyet and de Nabias. According to these observers, the organism is 30 to 36μ in length and 2 to 3μ in breadth, its flagellum is about 15μ long, and the pointed posterior end of the animal (from its tip to the kinetonucleus) is about 3μ in length. Bosc states that his specimens of this trypanosome are 26 to 28μ, or up to 30μ in length, and 2·5 to 3μ in width.

We have made careful measurements of the trypanosomes in films[1] prepared from our infected tame rabbits, and also in two films, kindly given to us by Dr. Petrie, prepared from his infected wild rabbits. The average dimensions of twenty trypanosomes from our films are—length of the posterior[2] end (*i.e.* from the tip of the organism to the kinetonucleus), 3μ; length of body, from kinetonucleus to the beginning of the free portion of the flagellum, 17·4μ; length of free portion of the flagellum, 8μ, giving a total length of 28·4μ. The corresponding average measurements of twenty trypanosomes on Dr. Petrie's films are—3μ, 18μ, 8μ; total length, 29μ. The length of the twenty organisms measured on our films varies from 27μ to 29·4μ, and that of those on Dr. Petrie's films from 28 to 31μ, but only two out of the twenty reached 31μ in length. Neither on Dr. Petrie's nor on our own films were specimens observed longer than 31μ, and in none was the free portion of the flagellum longer than 11μ, and in most of them it was only 7 to 10μ in length.[3] These measurements are, it will be noted, distinctly less than those given by Jolyet and de Nabias (see above). The width of *T. cuniculi*, from measurements made on the films above mentioned, is on an average almost exactly 2μ, in only one or two out of the many specimens observed was the diameter appreciably greater—namely, 2·7μ—except, of course, in the case of specimens in, or preparing for, division.

Petrie (1905[9], p. 193) pointed out that this trypanosome is not distinguishable microscopically from *Trypanosoma lewisi* (Kent), and after making a series of measurements of both these organisms we can entirely confirm this conclusion. In size and in the proportion of the various parts the two trypanosomes are closely similar, as may

[1] Ordinary dry films stained with Leishman's stain.

[2] Assuming the free portion of the flagellum to be anterior.

[3] A specimen of this trypanosome figured in Petrie's paper (1905, Pl. VIII. Fig. 4) is, according to the attached scale, about 37μ long.

be seen by reference to the average measurements of twenty-five specimens of *T. lewisi*,[1] which are as follows:—Posterior end, 3·8 μ; "body," 18·5 μ; free portion of flagellum, 7·7 μ; total length, 30 μ. The tapering posterior end of *T. lewisi* is *on an average* distinctly longer than that of *T. cuniculi*, but the individual variations in the length of this portion are such as to preclude its use as a distinguishing character, for they are almost as large in amount as the difference in the length of this region of the animal exhibited by the two species; for instance, specimens of *T. cuniculi* with posterior end as long as 3·6 μ, and of *T. lewisi* with posterior end only 3 μ long, are present in our films. In Laveran and Mesnil's treatise (English Edition, p. 104), *T. cuniculi* is stated to be "a little smaller [than *T. lewisi*[2]], and its post-centrosomic end is very short and slender," but, as we have shown above, the two organisms are very closely similar not only in their total length, but in the length of their "post-centrosomic end."

Although *T. cuniculi* is not distinguishable with certainty by its morphology from *T. lewisi*, these two organisms can be distinguished by their reaction on inoculation into rats. The blood of rabbits containing *T. cuniculi* was inoculated into guinea-pigs, rats, and mice by Bettencourt and Franca, and into guinea-pigs and rats by Bosc and by Petrie, but in every case trypanosomes could not subsequently be found in the inoculated animals. It has apparently not been always easy to transmit the organism to other rabbits; for instance, Bosc inoculated four rabbits, in regard to three of which the results were negative, while in the fourth case the trypanosomes appeared in small numbers only, and disappeared about the eleventh day after inoculation. Of five rabbits inoculated intraperitoneally by Bettencourt and Franca, two became infected, but the trypanosomes disappeared by the twenty-fifth day. In Petrie's experiments (1904[8]) ten rabbits were inoculated, five of which gave negative results, but four died. The cause of death could not, however, be established, but Petrie suggests that the trypanosoma blood was toxic in these cases. Petrie tried, but without success, to transmit the infection by inoculation from wild to tame rabbits.

In both wild and tame rabbits in which the infection has taken place either "naturally" or by artificial inoculation there are generally no morbid symptoms, the animals are usually apparently quite healthy. The only contrary observations are—(1) Possibly the cases recorded by Petrie (see above), in which four of the inoculated rabbits died; and (2) the cases observed by Nicolle in Constantinople, where the inoculated rabbits became cachectic and died. Jolyet and de Nabias, while stating that the health of infected animals does not seem to be affected, say that the parasite is more often found in rabbits which

[1] From films (fixed with osmic vapour) for which we are indebted to Professor Minchin.

[2] On p. 68 of the volume cited above, *T. lewisi* is stated to be 24 to 25 μ in length, but the two examples of this organism drawn in Fig. 16 (p. 103) average about 34 μ in length.

are wasted and ill, or in those which have suffered from diarrhœa, than in healthy rabbits. The period of persistence of the trypanosomes in the blood of infected animals seems to be usually comparatively short; in the infected rabbits observed by Bettencourt and Franca the trypanosomes were no longer demonstrable in their blood twenty-five days after they had been first noticed, but in one of Petrie's cases the parasites persisted for a period of ten months.

The rabbits in the blood of which we found *T. cuniculi* were, as already stated, three out of a batch of eighty-nine tame rabbits from the neighbourhood of Edinburgh. Although the blood of the other eighty-six rabbits was subjected to careful examination, no trypanosomes could be found, nor have they been observed in any of the rabbits subsequently acquired from the same sources.[1] The presence of trypanosomes in the blood of the three rabbits above mentioned was first noticed on 10th, 20th, and 21st May 1908 respectively. In the case of the first-named trypanosomes were demonstrable in the blood until 16th July (*i.e.* sixty-eight days), but in the two latter cases the typanosomes had disappeared from the blood by 2nd July (*i.e.* after forty-three and forty-four days respectively). In none of the cases was there any rise of temperature or any morbid symptom whatever; the animals were in excellent condition during the whole period the infection persisted, and one of them actually gained weight. The animals have been kept under observation until the time of writing (January 1909), and are still perfectly healthy. There has been no reappearance of the trypanosomes in their blood.

Experimental Work.

Two tame rabbits were injected intravenously with 4·5 c.c. infected citrated blood—equal parts of strongly infected blood and citrate solution; on the seventh day after inoculation numerous trypanosomes appeared in the blood, and for three weeks longer the organisms continued to be present in the blood in large numbers, after which time they gradually disappeared. During the whole period of infection the health of the rabbits was not in the least disturbed by the presence of the numerous flagellates in their blood; their weight was maintained, they fed well, and their temperature was normal. Two rats and two guinea-pigs were inoculated intraperitoneally, and two mice were injected subcutaneously, each animal with 0·5 c.c. blood from a strongly infected tame rabbit; these animals remained quite healthy, and no trypanosomes could be found in their blood, although they were kept under careful observation for a period of three weeks. Specimens of the blood of the infected rabbits, containing *T. cuniculi*, were kept in tubes at the room temperature, at 37° C., and in the ice chest at 8° C. The trypanosomes disappeared from the two former

[1] See, however, note at end of paper (p. 441).

sets of tubes (at room temperature and at 37° C.) in from one to three days, but after more than a week the organisms were actively motile in the tubes kept in the ice chest. Petrie [1] has already shown (1904, 1905) that *T. cuniculi* will maintain its motility unimpaired for at least a month when kept under these conditions.

Summary.

Trypanosoma cuniculi (Blanchard) has been comparatively seldom noticed; it has been previously recorded from the blood of either wild or tame rabbits in Elstree (Herts), Bordeaux, Montpellier, Thomar (Portugal), and Sardinia; [2] we now record the presence of this organism in tame rabbits obtained in and around Edinburgh. This trypanosome is, according to our measurements made on films prepared from infected wild and tame British rabbits, usually about 28 to 29μ long (not often exceeding 30μ in length); the free portion of the flagellum is about 8μ long, and the tapering "post-centrosomic" end is about 3μ long, the "body" of the animal being 17 to 18μ in length. *T. cuniculi* is morphologically very similar to *T. lewisi* (Kent), from which it is not possible to distinguish it microscopically; but the two organisms are distinguishable on inoculation into rats, which are quite refractory (as also are guinea-pigs and mice) to *T. cuniculi*. The health of the host is, according to our observations on five strongly infected rabbits, not appreciably affected by the presence of large numbers of *T. cuniculi* in the blood. The organism dies in from one to three days if the blood in which it is present be kept at room temperature or at any higher temperature, but it will remain actively motile for over a week (a month, according to Petrie) if the blood be kept at a low temperature (8° C.).

Note.—Since this paper was written, Dr. W. M. Scott has found another laboratory rabbit with trypanosomes of the same species in its blood. The rabbit was quite healthy.

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[1] To complete the summary of Petrie's observations, it should be added that he examined the newly born young from an infected female, but could find no trypanosomes, and that he cultivated *T. cuniculi* in rabbit blood agar, and obtained forms similar to those seen in cultures of *T. lewisi*.

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ON MENINGITIS ASSOCIATED WITH AN INFLUENZA-LIKE BACILLUS.[1]

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(Plate XL.)

Ever since M'Donald (1907–8 [18]), working in this laboratory, found, in certain cases of meningitis, organisms described by him as *Leptothrix* forms, a look out has been kept for similar forms. One such bacterium, which was isolated from a case of pyæmia associated with meningitis has been described by M'Donald and the author (1908 [24]). During the past year I have had the opportunity of investigating two other cases in which the organisms isolated were probably more closely related to those originally seen by M'Donald, and I have had access to a third case which probably belongs to the same group. These cases form the subject of the present paper.

Case 1.—For this case I am indebted to the kindness of Dr. John Thomson. It is that of a boy, æt. 19 months, whose only previous illness had been an attack of otitis media at the age of 13 months, from which he had completely recovered. The illness began with feverishness, drowsiness, temperature running from 102°–104°·5 F., stiffness of neck, head retraction, and cervical tenderness on moving head. On the tenth day the temperature ran from 103°·4 in the morning to 101°·8 in the evening; pulse, 140; respirations, 50 to 60; no signs of disease in ears, heart, lungs or abdomen, and urine normal; child quite conscious and intelligent, very sensitive to loud sounds, knee-jerks active, marked Kernig's sign. Lumbar puncture yielded $2\frac{1}{2}$ oz. faintly opalescent fluid, which escaped under considerable pressure. The sediment contained numerous Gram-negative bacilli. On the seventeenth day the child was almost unconscious; temperature, 104° F., respiration, 60; pulse, 164; general rigidity; lumbar puncture yielded 30 c.c. of fluid, and the same amount of Flexner's anti-meningococcic serum was injected. A temporary fall of temperature followed, but no improvement resulted and the child died on the twentieth day.

The second sample of cerebro-spinal fluid was sent to me for examination. It had been withdrawn under strict aseptic conditions, and Dr. Thomson stated

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that it was more turbid than the specimen first withdrawn. When stained by Gram it was found to be rich in leucocytes, the prevailing type being the polymorpho-nuclear. It contained a number of Gram-negative bacilli, apparently all of the same kind. These were about 0·3 to 0·4 broad, and varied much in length, some long forms being present. No other organisms were detected in the fluid.

Cultures were made in agar, serum, bouillon, glucose bouillon, and blood-smeared agar (in the latter both aerobic and anaerobic tubes). Growth only took place in the last-named medium in the aerobic tube. It was very sparse, and consisted of small transparent colonies which developed best in the little shreds of blood clot present. These were found to consist of bacilli identical with those seen in the films from the cerebro-spinal fluid, and showing very frequently long filamentous forms.

Case 2.—For this case I am indebted to Dr. Claud Ker, of the Edinburgh City Hospital. The patient, an infant, was admitted to the hospital on 29th October 1909; notified as a case of cerebro-spinal fever. Lumbar puncture the same night yielded nothing, but on 30th October about 5 c.c. of a turbid fluid were obtained by Dr. John Ritchie, bacteriologist to the hospital. On this day also a purulent otorrhœa was observed. Part of the fluid along with a serum culture was sent to me by him on 1st November. The child died on 31st October, and no post-mortem was obtained. The fluid sent was found, on microscopic examination, to show chiefly a polymorph exudation in which there were a number of Gram-negative bacilli,—nothing resembling meningococci or other organisms being seen. Similar bacilli were present in the serum culture which had been made by Dr. Ritchie.

Cultures were made on all the ordinary media, and on rabbit-blood agar both aerobically and anaerobically. Growth appeared only on the latter medium, and was most vigorous on the aerobic tubes. Its characters were identical with those of the cultures from Case I.

Case 3.—This case was brought under the notice of Dr. Stuart M'Donald and myself by Dr. Claud Ker at the time we were working up the case of pyæmia already alluded to. It is that of a child, æt. 5 months, who ten days previous to admission to the City Fever Hospital was seized with convulsions, repeated three days later; there was also frequent vomiting. On admission there was high fever, marked head retraction, neck rigidity. Kernig's sign was present, the child was conscious, and there were no skin hæmorrhages. On the next day (eleventh of illness) head retraction was still marked; breathing rapid, 60; temperature, 103° F. On the twelfth day, convulsions, eyes rolling, fontanelles tense, respiration 84, temperature 106° F., and death in convulsions; no post-mortem obtained. The child had had discharge from the ears.

Lumbar puncture was performed on three successive days; the fluid was slightly turbid, and contained polymorph cells. On the first occasion there were slender bacilli present (Plate XL. Fig. 3). A pure culture of a leptothrix bacillus is noted as having been obtained on Löffler's serum, but subcultures failed to yield growths. No meningococci were seen microscopically, nor did such appear on culture tubes. The serum of the patient did not agglutinate either the meningococcus or Batten's coccus of posterior basal meningitis.

A film from the cerebro-spinal fluid was given to us by Dr. Ker, and it contains bacilli resembling in every way the bacilli isolated by me from Cases 1 and 2.

So far as can be seen, the organisms isolated from the Cases 1 and 2 are identical in microscopic, cultural, and general pathogenic properties. It is a non-motile bacillus which stains with ordinary dyes, and is negative to Gram. In young cultures it is from 0·3 to 0·4 μ broad,

but varies much in length. Sometimes the prevailing length in a culture is from 0·5 to 1·3 μ, but even in such cultures long forms measuring from 10 to 15 μ are met with (Plate XL. Fig. 1). These are often irregularly bent. In older cultures these long forms may be a prominent feature, and the filaments often double on themselves. Generally speaking, the protoplasm of the organism stains uniformly, but sometimes intensely stained particles may be observed, especially at the ends of the long individuals. In older cultures, say from five to ten days, involution forms are prevalent, and these assume many aberrant shapes,—wavy spirals, exaggerated commas, elongated flask shapes, long filaments with speckled protoplasm, etc. (Plate XL. Fig. 2). Nothing resembling spore formation has been observed.

As has been indicated, when the cerebro-spinal fluid was used as the infective material, free growth was alone obtained on agar smeared with rabbit or human blood,—the latter perhaps giving the better result. On ordinary agar infected from the same source, either no growth resulted or only a few weak colonies which soon died out. When the organism has, however, been cultivated on blood agar for some months subculture on ordinary agar is more successful, and further subcultures can be carried on for some weeks; the colonies are, however, almost invisible to the naked eye, and smears show the bacilli to be mostly very small individuals. The most characteristic growth is that on blood agar as described. After twenty-four hours at 37° C. minute dewdrop-like colonies are observable, which on further incubation do not increase in size. At room temperature no growth takes place, but when growth is started at 37° C. the cultures can be kept for a day or two at room temperature without dying. Growths kept at 37° C. are alive after at least eighteen days, but subcultures made after long intervals show a more sparse growth than those made from young cultures. On other media a growth has, hitherto, not been obtained.

Inoculation Experiments.—1. A *Rhesus monkey* had two blood-agar cultures injected into the lumbar region of the spinal canal. It died within eighteen hours, and the organism was recovered by culture from the surface of the spinal cord and from the base of the brain. The whole of the spinal cord and the base of the brain were in a state of commencing meningitis. In both situations a greyish exudation was beginning to appear. Smears from the base of the brain showed an abundant cellular exudation,—the chief cells present being polymorphs; there were also some large mononucleated cells with a rather small nucleus and slightly acidophil protoplasm. Some of these mononucleated cells and many of the polymorphs contained in the protoplasm phagocyted bacilli, some long forms being included. There was a tendency to karyorrhexis in some of the polymorphs. There were an enormous number of free bacilli in the smears, practically all of these being short forms. In films made from the surface of the spinal cord there were enormous numbers of large mononucleated cells, derived, apparently, from the ependyma, the prevailing type being a large oval cell with oval nucleus often placed at one end. There was a relatively small number of polymorphs, which, however, often contained bacilli. The specimens were crowded with free

bacilli. It may be said that the inoculation of the monkey was carried out with cultures from Case 1 a few days after the organism had been isolated from the spinal fluid. While the death of this animal was probably chiefly due to the toxic effects of an overdose, there is no doubt that the organism had multiplied in its body and was commencing to cause a meningitis.

2. *Guinea-pigs.*—Pressure of other work prevented further inoculation experiments being carried out until blood-agar subcultures from Case 1 were about five months, and from Case 2 about two months, distant from their isolation from the human body. After the lapse of these periods it was found that both organisms were pathogenic to guinea-pigs, one and a half cultures injected intraperitoneally killing these animals in thirty-six hours. The organism was isolated from the peritoneal cavity and from the heart blood. There was a purulent peritonitis, the exudation being scanty in amount and glairy in character. It was richly cellular, the prevailing cell being the mononuclear; many of these were obviously derived from the blood, but there were also many large elements often oval with an oval nucleus derived from the peritoneal endothelium. Sometimes a good many free bacilli were present in the peritoneal fluid, and there was a slight degree of phagocytosis. It may, however, be stated that when only short forms of the organism are present it is often difficult to be certain both of the presence of phagocytosis and of free bacilli.

On the pathogenicity being tested in guinea-pigs about two months later it was found impossible to infect adult animals either intraperitoneally or hypodermically, but a young guinea-pig of about 300 grms. succumbed to intraperitoneal injection of two cultures after an illness of three days. There was a hæmorrhagic peritonitis, the exudate from which had the characters already noted, and the causal organism was recovered from the peritoneum and heart blood.

3. *Other animals.*—At the time the first guinea-pigs were inoculated rabbits received three cultures intravenously without effect. A similar result followed hypodermic inoculation. Intravenous injection of the organism which was fatal to the small guinea-pig was not effective when two cultures were introduced intravenously into a young rabbit of 7 weeks old. Intraperitoneal and subcutaneous injection of the organism in a dose of one culture had no effect on mice.

It was very unfortunate that in none of the cases described was a post-mortem examination obtained, but it may be said that from two cases (1 and 2), which presented clinical characters recognised by two most competent observers to be those of acute cerebro-spinal meningitis and in which the cerebro-spinal fluid presented the cytological appearances of fluid from non-tuberculous cases, the only organism detected both by microscopic and cultural methods was a bacillus which had the morphological and cultural characters of the influenza bacillus, *i.e.* the organisms isolated were non-motile, Gram-negative bacilli, in size corresponding to the influenza bacillus and presenting the further characteristic of this organism in only growing on media containing blood. The bacillus was further capable of causing pathogenic effects on guinea-pigs and the monkey.

From the time of the first of the great modern influenza epidemics in 1889–90 meningitis has been recognised as an occasional complication or sequela of the disease. Clinically, the condition often cannot be differentiated from the so-called post-basal type, but in a good many

cases restlessness and wild delirium have been more marked than usually is the case in ordinary acute meningitis. Sometimes recovery has occurred, but usually the disease has had a fatal result.

Influenzal meningitis has been reported on by Leichtenstern (1890 [17]), Kuskow (1895 [15]), Hunermann (1899 [13]), and others. It is difficult to form an estimate of the relative frequency of meningitis as a complication of influenza, but Tadesko (1907 [26]), in 1227 post-mortems performed in Vienna between 1896 and 1906 in which influenza bacilli were found in the bronchi, records ten cases where the clinical diagnosis was meningitis. Of these, in four cases no meningitis is recorded post-mortem, in two there was simple meningitis, in two a purulent meningitis, in one tuberculous meningitis, and one is put down as septicæmia. The results of bacteriological examination of the brain are not recorded, but in one case the influenza bacillus was found in the lumbar puncture fluid, and in another it was present in pus in the sphenoidal sinuses. Among the observations on influenza meningitis considerable attention has been devoted to those of Pfuhl (1892, 1897 [23]; see also Beck, Ghon, *loc. cit.*[1]). This observer has, according to Ghon (there is one of Pfuhl's papers to which I have not had access), described fourteen cases of meningitis occurring during epidemic outbreaks of influenza. In several of these the meningitis presented the appearance, post-mortem, of an intensely inflammatory hyperæmia accompanied by encephalitis, but in some cases there was a purulent condition on the surface and at the base of the brain, and in one an abscess of the cerebellum occurred. In all the series Gram-negative bacilli resembling the *B. influenza* were found and often isolated, and in one case similar bacilli were found in the liver, spleen, and kidney. Unfortunately, in most of the cases other bacteria—cocci and "putrefactive organisms"—were also observed, and this fact has caused several critics to doubt Pfuhl's deductions that the influenza bacillus was the direct cause of the meningitis. In a few of his cases, however, there is little doubt that such a bacillus was present alone in the central nervous system.

Apart from the occurrence of meningitis complicating a definite influenza a number of instances have occurred, of which the chief are recorded below, in which meningitis has been associated with influenza-like bacilli. Those cases have mostly been sporadic in their distribution. To this group my own cases belong, as at the times of their occurrence influenza was not present in Scotland in an epidemic form.

Thus Cagnetto (1904 [3]) records the occurrence in Venice of the cases of two children, æt. 12 and 13 months, who died after illnesses of sixteen and ten days respectively. During life the symptoms were those of meningitis, and after death there was found an abundant fibrino-purulent exudation in the subdural space and in the pia arachnoid, especially over the vertex and also in the base and extending down the spinal cord, where it was specially marked in the lumbar region. The brain substance was œdematous and the ventricles were distended with exudate. In both cases beads of pus were found in the tympanic cavities. In both cases the naso-pharynx was normal. In one case there was catarrh of the larger bronchi, and also some recent fibrinous perisplenitis. In the other case there was an acute parenchymatous nephritis. In preparations of the meningeal exudate and the spinal fluid influenza-like bacilli were seen, and these were obtained in pure culture on aerobic pigeon-blood agar and on no other medium.

Cohen (1909 [5]) records three fatal cases from Brussels, occurring in children of 4 months, 1 year, and 8 months. All presented the clinical picture

of meningitis. In one there also existed a secondary broncho-pneumonia with a purulent pleurisy; in a second there was a similar pneumonia and an empyema of the left maxillary antrum; in the third case a broncho-pneumonic condition was diagnosed before symptoms of meningitis appeared, and there was a suppurative arthritis of the left wrist. In the first case alone was a post-mortem obtained; this confirmed the diagnosis formed during life, and revealed a thick purulent exudate over the brain and cord. From the lumbar fluid taken during life a hæmophilic influenza-like organism was recovered. In the cerebro-spinal fluid of the other two cases microscopic examination revealed a small bacillus, often, however, occurring in filaments. Culture on rabbit blood agar yielded a pure culture of an organism identical with that obtained from the first.

Mya (1902 [20]) records three similar cases in children whose ages were 8 months, 1 year, and 9 months. Two of these were fatal, while one recovered with a hemiplegia; in one of the former there was in addition a suppuration of the right shoulder-joint, and in the latter a suppurative otitis was observed in the later stage of the disease. These cases are interesting in that they all occurred within short intervals of each other, and although influenza was not epidemic in Florence at the time there was an epidemic of measles during which four cases of lung affection were observed in which the influenza bacillus was isolated from the chest lesions; moreover, about the same time this bacillus was also found present in lung lesions observed in children who had not suffered from measles.

Peucker (1901 [21]) records the case of a child of 5 months which died from pneumonia and meningitis. The diagnosis was verified post-mortem, there being a lobular pneumonia, with bilateral fibrinous pleurisy, purulent meningitis with encephalitis, and suppurative otitis media. An influenza-like bacillus was recovered from the meningeal exudate along with a few staphylococci, and it was also present with the pneumococcus in the lungs and in the pus from the middle ear. Peucker considered the meningitis secondary to the lung condition or to that of the ear,—which, could not be determined. Influenza was not prevalent at the time.

Hecht (1903 [12]) has recorded, in a child of 2 years, a similar case of influenza pneumonia and suppurative meningitis with an otitis, which, however, apparently developed later than the other conditions. An influenza-like bacillus was isolated from the lumbar fluid.

Similar cases have been reported by Langer (1901 [16]) in a child of 9 years, Douglas (1907 [7]) in a child of 10 months, Haedke (1897 [11]) in an adult, and by Ghon (1902 [10]) in a child of 8 months. Probably the case reported by Milligan (1908–9 [19]) was of the same kind.

Dudgeon and Adams (1907 [8]) have published a case of multiple arthritis occurring in a child of 10 months, with a terminal meningitis in which from the elbow joint, hip joint, spleen juice, and lumbar fluid the influenza bacillus was obtained. There was no history of any illness like influenza having been suffered from.

Somewhat similar is the case of Slawyk (1899 [25]), where in a boy of 9 months old death resulted from a pyæmic affection of one wrist, one ankle, and one knee, with purulent bronchitis and a purulent meningitis. A hæmophilic influenza-like bacillus was isolated from the lumbar puncture fluid, from the pus of the wrist and the finger blood, while in the pus from the ankle and in the lungs microscopically similar bacilli were seen.

The cases recorded may for convenience be classed in two groups: firstly, those occurring during the prevalence of epidemic influenza, and those not apparently connected with such outbreaks. With regard to the former, there can be no doubt that meningitis does occur as a sequel

or complication of the disease, and if Pfuhl's cases be accepted as due to the influenza bacillus and not to the other organisms found by him, it may be that a rather special type of meningitis is to be looked for in influenza in which an intensely hyperæmic condition associated with areas of softening in the brain and encephalitis are characteristic features. Even in cases occurring during an epidemic, however, purulent meningitis may be observed. It is with the second group of cases, which are of a more sporadic character, that we are chiefly concerned. From the evidence which has been detailed above there can be no doubt that from time to time there have occurred in Germany, Austria, Italy, Belgium, and Britain cases of purulent meningitis in which the only organism present has been a bacillus closely related to that of influenza. In a majority of the cases the brain has apparently been the internal organ primarily affected, though, in some, lung symptoms have manifested themselves at a date anterior to those of meningitis. Very generally, post-mortem signs of lung inflammation or of purulent bronchitis have been found. There are three specially outstanding features of these apparently sporadic cases: firstly, the fact that this form of disease chiefly attacks children of very tender years: out of the sixteen cases recorded above (including those of the author) in which the age has been given, twelve took place in children of one year or under; secondly, the condition is extremely fatal,—only in one case—one of those recorded by Mya—has recovery been noted; thirdly, in several cases (Dudgeon and Adams, Ghon, Slawyk, Cohen) the brain affection has been part of a pyæmic process. A further point of note is the frequent association of the meningitis with suppurative otitis media or with suppuration of the frontal sinuses or the maxillary antrum. The significance of these suppurations is difficult to estimate. They might be indicative of the meninges having been affected secondarily to an ear condition. On the other hand, the occurrence in certain cases of evidence of pyæmia might point to both the otitis and the meningitis arising from some common source elsewhere than in the head. In certain cases the ear symptoms have preceded the meningitis,—in one of the author's cases by some months; in others the otitis has developed during the course of the meningeal affection. With regard to the latter cases, the difficulties inherent in the recognition of the earlier signs of ear inflammation in a young child sickening for a more serious affection may be borne in mind, and in this connection Hecht's case is interesting, as in it, early in the illness, the presence of a simple otitis was detected which later developed into a suppurative condition. The only cases where the naso-pharynx and accessory parts were found post-mortem to be free of disease were the two recorded by Cagnetto, that of Ghon, and perhaps of Langer. In connection with the possibility of an influenzal meningeal infection arising through the ear, it may be noted that Kossel (1893 [14]), in 108 post-mortems, on children of 1 year and under

found eighty-five cases of middle ear disease, and in half of these an organism which was looked on as the influenza bacillus was present. Taking into account all the available facts, a provisional opinion may be expressed that in many cases—perhaps in the majority—the meningitis resulted from a spread of an infective condition from the naso-pharynx. In certain cases, however, the pathway of the meningeal infection is possibly different. The occasional occurrence of signs of pneumonia early in the disease may indicate that the organisms first gained entrance to the lungs, and thence found their way by the blood stream; as bearing on the possibility of this taking place, it will be indicated later that an escape of influenza bacilli into the circulation is probably more frequent than has hitherto been supposed.

When we come to consider whether the organism isolated from the sporadic type of meningitis is identical with the organism found in ordinary epidemic influenza, we are faced with a question of great complexity. In the first place, it must be remembered, with regard to the influenza bacillus, that we are dealing with an organism whose causal relationship to the disease from which it was first isolated rests on indirect and incomplete evidence. Secondly, organisms of a similar kind have been obtained from conditions of the respiratory tract which otherwise did not present clinically the typical influenzal syndrome; further similar organisms have been described in connection with affections—especially of the eye—which by themselves have been looked on as specific entities unconnected with influenza. The former conditions perhaps present the least difficulty, as, assuming the influenza bacilli to be the cause of influenza, one effect of the recent great epidemics must have been to distribute the organism very widely in the world,—often it may be in a condition of altered virulence in which it is only capable of producing modified effects. Taking all the facts into account, however, we must be prepared to face the position that the influenza bacillus is only one of a group of allied organisms. The differentiation of the members of the group must be a matter of great difficulty, in consequence of their capacities to grow under saprophytic conditions being limited and of the somewhat limited power they possess of originating disease in animals. We must therefore inquire whether we are at present justified in attempting to differentiate organisms, isolated from different sources, from one another.

The influenza bacillus, as originally described by Pfeiffer, was characterised: (1) By its relations to the lesions of the disease; (2) by its cultural and morphological appearances; and (3) by its pathogenic effects in animals.

1. With regard to the relations to the disease of which it is supposed to be the cause, Pfeiffer's original view (*e.g.*, 1893 [22]) was that the bacillus gives rise to a catarrhal process in the respiratory tract; that it is confined in its habitat to the free surfaces of that

tract, and that it gives rise to constitutional effects by toxic action. From the very first, controversy arose as to whether an escape of the organism into the blood ever took place. In this connection it may be pointed out that in his original work Pfeiffer used comparatively small amounts of blood (10 to 20 drops) for his investigations. Other observers (*e.g.* Ghedini 1907 [9]), using larger amounts (20 to 30 c.c.), have succeeded in demonstrating the organism in the blood, and by similar methods also in the spleen juice. Here such cases as those of Pfuhl (*supra*) form a connecting link between the influenza bacillus as a toxic agent and as an originator of septicæmic processes. Pfuhl's cases, as has been noted, occurred during influenza epidemics, and in them the organism was found in the inflammatory lesions in the central nervous system. The observations are significant even when it is granted that other organisms appear also to have been present.

2. The classical type of morphological and cultural reactions presented by the influenza bacillus has already been described. The question has been raised as to whether there exist other allied forms, all of which conform, more or less, to the type,—especially in being essentially hæmophilic,—but which present sufficient differences to justify their being recognised as in reality merely allied species. It is beyond the scope of the present paper to consider the types isolated from apparently distinct clinical entities such as specific forms of conjunctivitis, and therefore attention will be alone directed to the relationships of the organisms isolated from septicæmic and pyæmic conditions such as meningitis. From this narrower standpoint interest centres chiefly round the question of the existence of what Pfeiffer [1] denominated the "pseudo-influenza bacillus." This organism was isolated by Pfeiffer from the lungs of three cases of pneumonia occurring, secondarily to diphtheria, in children during the waning period of an influenza epidemic. It had the general characters of the true bacillus, but was thicker and—what from our present standpoint is of great interest—showed a great tendency to the formation of long filaments. According to Beck (1903 [2]), it has the further character of being non-pathogenic to animals. Here again, however, there has been a tendency on the part of numerous observers to question the existence of differences between the true and false bacillus. Thus Albrecht and Ghon (1901 [1]), after a careful consideration of the evidence, conclude that in cultures the true bacillus not only shows considerable variations in thickness, but also may develop filamentous forms to as great a degree as may be observed in the alleged pseudo-type. The general conclusion here must be that cultural and morphological methods do not serve for the differentiation of strains yielded by cases of influenza from strains derived from other, and especially from septicæmic, cases.

3. When we consider pathogenic effects in animals as a possible

[1] *Loc. cit.*

means of differentiating the true influenza bacillus from allied organisms, we are at the outset met with difficulties. On the one hand, the number of investigators who have studied the pathogenic properties of strains isolated from influenza cases is relatively small, and on the other not only do the effects produced by organisms coming from other disease manifestations differ, but the same strain may, at different periods, show different pathogenic powers. In Pfeiffer's original papers the position is taken up that the influenza bacillus is only pathogenic to apes and rabbits, the paths of infection used by him having been the trachea and lungs in the former and the intravenous method in the latter. In the rabbit the fatal effects were chiefly attributed to toxic action, as dead cultures had much the same effects as living. Cantani (1896 [4]), however, and also Delius and Kolle (1897 [6]), brought forward evidence, based on the effects of small doses of virulent cultures (derived from influenza cases), that a true multiplication of the organism occurred in rabbits. Cantani produced purulent pleurisy by intraperitoneal infection and abscess formation when the bacteria were injected subcutaneously. In both researches it was further shown that guinea-pigs could be infected and suffered from purulent or sanguino-purulent peritonitis, with in certain cases (Delius and Kolle) evidence of septicæmia,—the organisms being recovered by culture from the blood. Thus it has been shown that strains derived from influenza cases are pathogenic to apes, rabbits, and guinea-pigs, and that they are capable of giving rise to pyæmic and even septicæmic processes. Cantani also produced meningitis by intracranial injection in rabbits; here the brain was hyperæmic, often presented punctiform hæmorrhages, and the ventricles contained an exudate, sometimes purulent. Turning to the results of inoculation of influenza-like bacilli derived from sources other than ordinary influenza, we have a number of observations. Albrecht and Ghon, working with a hæmophilic organism isolated from a case of subcutaneous phlegmon which died with signs of septicæmia, found it pathogenic for rabbits, but not for guinea-pigs or mice. Several experiments have been made with organisms isolated from meningitis cases. Thus the organisms from two of Mya's cases were pathogenic for rabbits. Cagnetto also found that rabbits succumbed with septicæmia and peritonitis to the intraperitoneal injection of the ventricular exudate from one of his cases, but subcutaneous, intravenous, and subdural infection failed, as likewise did the inoculation of guinea-pigs, mice, and a cat. Cohen found the organisms from his case to be pathogenic for rabbits and guinea-pigs, in both animals septicæmia with purulent serous inflammations being observed. In my own cases I found the organisms pathogenic for guinea-pigs, but not for rabbits and mice, and with one strain a fatal illness was originated in a monkey. Slawyk in his case also found the meningeal exudate and blood-bouillon cultures from the same to be pathogenic to guinea-pigs.

The type of pathological changes experimentally originated by organisms derived on the one hand from influenza causes, and on the other from meningitic or pyæmic is the same. There either occur inflammations of an intensely hyperæmic kind with a tendency to punctiform hæmorrhages, or inflammations going on to suppuration are found. Any differences observed are probably explained by differences in the path of infection or by differences in the stage in the disease at which the animal succumbs.

The general conclusions here may be said to be: (1) That even in influenza evidence accumulates that the organism is not confined to the free surfaces of the respiratory tract, but may also be found in the blood and in the organs of the body; (2) cultural methods are insufficient to differentiate influenza bacilli isolated from influenza cases from similar bacilli coming from other disease conditions; (3) the pathogenic effects produced in animals by strains coming from influenza cases and those from other sources are similar. Perhaps, in the future, information as to the existence of differences may come from the study of the toxic effects of different strains or from the study of their serum reactions.

At present the justification for saying that a group of allied forms exists rests on the fact that they have been derived from clinical conditions of apparently different characters, *e.g.* influenza, conjunctivitis, pyæmia. From the bacteriological standpoint there is not, at present, sufficient evidence for the organisms being differentiated from one another. It is thus obviously impossible to dogmatise as to whether the organism isolated from the sporadic cases of meningitis with which we are here concerned is identical with the organism occurring in the respiratory tract in influenza.

In the meantime it is sufficient to say that there exists a group of cases which often presents pyæmic characters, in which meningitis is a very common occurrence and in which the only organism present is a hæmophilic bacillus having the characters of the influenza bacillus. These cases are especially common in children under two years of age in whom the disease is of a very fatal type. Against the causal organism here being the true influenza bacillus might be brought forward the statement, frequently made, that children are not very susceptible to influenza in its usual form. It may, however, be said that this supposed insusceptibility requires further investigation before its existence can be admitted. It may also be said that, apart from the occurrence of true influenza in children, further investigation is required of the infections of the naso-pharynx both in children and adults, which are associated with the presence of organisms of the influenza type.

The causal organism in cases such as those described in this paper may be very easily overlooked. In the first place, unless it is present in its filamentous form it may be missed if ordinary staining methods,

especially the Gram method, be alone applied. The organism is so small that its presence does not obtrude itself on the observer's notice. The difficulty here will often be got over if, as Pfuhl recommends, films of pus or of cerebro-spinal exudate be stained with carbol-fuchsin, one part in four of water, the staining being aided by gentle heat and applied for from ten minutes to a quarter of an hour. Secondly, the presence of the bacillus will almost certainly be overlooked unless the material containing it be inoculated on to a blood medium, preferably agar smeared with pigeon, rabbit, or human blood. If this method of investigation be not followed it is easy to class cases of this type of bacillary meningitis as cases of meningococcal meningitis in which it has been impossible to isolate the meningococcus.

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DESCRIPTION OF PLATE XL.

Fig. 1.—Film from young culture from Case 1, showing both small forms and the occurrence of filaments. (Gram.) (× 1000.)

Fig. 2.—Film from old culture, showing beading of protoplasm and involution forms. (Gram.) (× 1000.)

Fig. 3.—Film from cerebro-spinal fluid, showing long filaments. (Carbol-thionine-blue.) (× 1000.)

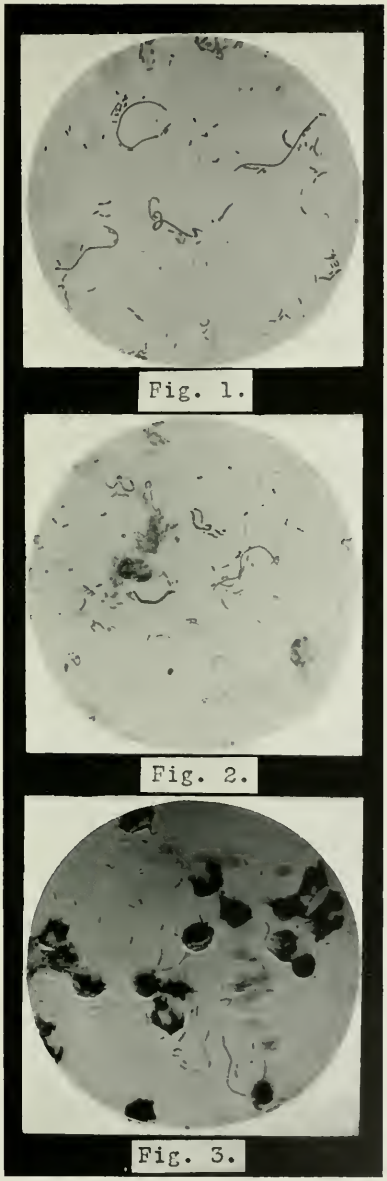

Fig. 1.

Fig. 2.

Fig. 3.

THE PATHOLOGY AND ETIOLOGY OF MONILETHRIX.[1]

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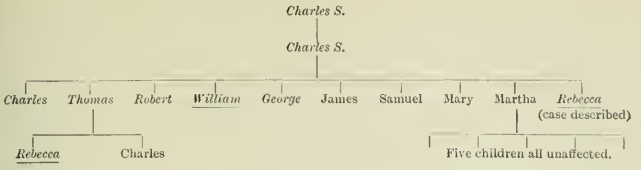
(Plates VIII. and IX.).

Monilethrix is a condition which was first described by Walter G. Smith twenty-nine years ago, and since then only about forty observers have recorded cases of the disease, and only four of these observers have examined the condition histologically. The etiology is still doubtful, and it was with the view of trying to throw some light on it that the following investigation was undertaken. I am indebted to Dr. Norman Walker for kindly allowing me to put these cases on record.

Case.—R. S., a schoolgirl, æt. 11, was seen as an out-patient at the Royal Infirmary in August 1907. The mother stated that since the age of nine months the patient's hair had never grown properly. On looking at the scalp one was at once struck by the appearance (Plate VIII. Fig. 1). The whole scalp was almost bald except for very short broken off and twisted hairs, which at first sight suggested a generalised tinea capitis. But on looking carefully it was seen that some of the hairs were broken off level with the surface, others were twisted and bent in different directions, whilst a few were several inches long. The long hairs were few in number and looked quite normal. The short broken hairs even with the naked eye could be seen to have a definite nodular appearance presenting alternate thick and thin areas. The scalp was not scaly, and more or less all over, but chiefly posteriorly, it was rough like a nutmeg-grater owing to the projection of the hair follicles. These follicular projections were reddish in colour and surmounted by a horny mass from most of which stumpy nodular hairs projected. The scalp alone was affected, the eyebrows, eyelashes, and body hairs being quite normal. There was a marked prominence of the follicles with horny plugs (keratosis pilaris) on the backs of both upper arms. The skin was otherwise quite healthy and the nails were well formed. The family history elicited was very remarkable, the child's grandfather, father, five brothers and one niece all being affected in the same way. The following genealogical table shows which members are affected.

[1] Received February 8, 1909.



Those whose names are in italics were not seen, but the patient's mother said that their hair was exactly like the patient's. Those whose names are in italics and underlined I have myself examined and found to be affected. In all the cases the peculiarity of the hair had been noticed soon after birth and had lasted all the life.

Hairs from the three cases seen were examined microscopically in Canada balsam (Plate VIII. Fig. 3). All the hairs are not similarly affected. Some do not show any nodes at all and are as a rule less pigmented than are the nodose hairs. Others look perfectly normal for part of their length, and then show one or more constricted areas at irregular or regular intervals. Those hairs which are most affected are curved and show a quite regularly beaded appearance. The nodes are about the thickness of the normal hairs and show cuticle, cortex, and pigmented medulla. The medulla, however, is not present in the nodes on all hairs. The internodes are about one-fourth the thickness of the normal hair and show a slightly thickened cuticle and a condensed central part with no pigment, and are evidently composed of the cortical substance of the hair. The node therefore is to be regarded as the normal part of the hair and the internode the abnormality. Many of the hairs show a partial or complete fracture at the internodes. Some only show a tearing of the cuticle, others are broken completely across, and where that occurs the ends are usually frayed out in a brush-like manner (Plate VIII. Fig. 3, *d.*) as in trichorrhexis nodosa. When pulled on, the hairs always break at an internode, never through a node. It was found quite impossible to remove the hairs by the root as they always broke off at an internode somewhere near the outlet of the follicle, leaving the rest of the hair in the follicle. No trace of any bacteria or fungus was found on any of the hairs.

A small portion of the scalp from the back of the head just above the occipital protuberance was excised. It was fixed and hardened in alcohol, embedded in paraffin, cut in serial sections and stained in various ways.

Owing to the hair follicles not running in a straight direction only one follicle was cut in all its length (Plate IX. Fig. 4). This section shows that the constrictions are present in the hairs from the very beginning of their growth. In Plate IX. Fig. 4 the hair bulb has become mechanically displaced, but from an examination of that hair and those seen in other sections (Plate IX. Figs. 5 and 6) it is

seen that starting a short distance above the bulb there are nodes and internodes at regular intervals. From the hair bulb to the surface there are from four to five nodes inside each follicle. All the nodes and internodes are at exactly the same distance from the bulbs. The first constriction is seen whilst the hair is still very cellular, and in some of the sections shows an appearance like a green-stick fracture at that point (Plate IX. Figs. 5, 6, and 8). The hair follicles themselves do not show any marked abnormality. The connective tissue surrounding the follicle shows nothing of note. The layers of the external and internal root sheaths are all well developed. The outer border of the external root sheath is quite straight, but its inner border shows a slight tortuosity corresponding to the thick and thin parts of the hair. There is no evident abnormal proliferation of the cells, but opposite the constricted part of the hair the sheath is broader owing to the cells being more spread out, whereas opposite the thick part of the hair the cells are more pressed together. The inner root sheath is of normal thickness in all its length but slightly sinuous, corresponding to the curves in the outline of the hair. The outer layer of the internal root sheath (Henle's layer) looks quite normal. The inner layer of the internal root sheath (Huxley's layer) is composed of cells with large oval nuclei, and clear somewhat hyaline-looking masses are seen with the oil-immersion lens inside the cells. These layers are closely applied to the hair opposite the nodes, but at the internodes there is a considerable space which is bridged across by the very loosely arranged cells of the cuticle of the root sheath and hair (Plate IX. Fig. 8).

The sebaceous glands are conspicuously few in number and very small. The majority of the hair follicles have no sebaceous glands associated with them. Another marked feature of the sections is the great cellular infiltration around the blood vessels of the corium. This infiltration consists of small mononucleated cells with deeply stained nucleus and scanty protoplasm and sections stained with Unna's polychrome methylene-blue show a large number of mast cells (Plate IX. Fig. 9).

Many of the follicles also show a marked widening of the orifice, which is filled with a horny plug (Plate IX. Fig. 7). In relation to these follicles there is also a cellular infiltration similar to that seen around the blood vessels. The sweat glands are fairly well developed. The elastic tissue is rather poorly developed. In sections stained by Pfeiffer's method with carbol fuchsin no organisms were found.

As stated at the outset this condition was first described by Walter G. Smith in Britain in 1879 and in the same year Luce in Paris wrote a thesis on the subject. The terms node and internode, as applied to the appearance of the hairs, although convenient, are misleading because, as has been pointed out by repeated observers, the node is the normal part of the hair and the constricted part the abnormality. Lesser called the condition ringed hair and aplasia pilorum intermittens; but the latter name implies a theory as to its causation, and the former is apt to lead to confusion with the other condition

called by the same name where there are alternate dark and light rings on the hairs without any alteration in the diameter of the hairs. M'Call Anderson's name of hereditary trichorrhexis nodosa is not a good one, as the trichorrhexis is a secondary and purely accidental phenomenon. The best name is that given to the condition by Crocker, namely, monilethrix, as it describes the shape of the hairs without implying any theory.

In our three cases the scalp alone was affected, as is the rule, but in Smith's ([43]) case the axillary hairs and one hair in the pubic region were found to be affected. Payne ([37]), Arndt ([4]), and Abraham ([1]) report cases where the eyebrows and lashes and Gilchrist ([23]) and Ruggles ([40]) cases where the hairs in patches on the lower limbs were similarly altered.

In our cases the disease had lasted nearly all the life, beginning in each case at from six to nine months of age. This is the usual time of commencement, the child being born with apparently normal hair which falls out during the first year of life and never grows again properly. In Smith's ([44]), Unna's, Gilchrist's ([23]), and Francis's ([22]) cases the condition did not commence till puberty. In Smith's and Gilchrist's cases no reason could be found, but in Unna's case mental shock (grief) preceded the commencement. In Francis's case the hair fell ont during the second year of life and grew in again white, gradually becoming darker in colour till at fifteen, after influenza, the monileform hairs were first noted. The condition once established has never been known to disappear except in Gilchrist's case, where the patches on the limbs disappeared after persisting for nine years.

Heredity plays an important rôle. In our cases nine members of the family were affected in four generations. Sabonraud ([41]) reports seventeen cases in five generations, M'Call Anderson fourteen cases in five generations, and Hallopeau and Lefevre ([25]) five cases in two generations. But heredity does not seem to be essential as Abrahams, Fox, Francis, Gilchrist, and Guszmann ([24]) could find absolutely no family history of the disease in their cases.

The association between monilethrix and keratosis (lichen) pilaris is very close, Török ([49]) going so far as to class them as one disease. On the scalp nearly all the recorded cases were associated with a hyperkeratosis of the follicles, and keratosis pilaris is recorded on the limbs of monilethrix patients by Bering ([11]) Besnier, Brocq ([16]), Tennessen ([47]), Hallopean and Sabourand. But here again the conditions are not necessarily combined.

In only four cases has a microscopical examination of the skin been made, namely, by Beatty and Scott ([6]), Bering ([11]), Guszmann ([24]), and Ciarocchi ([19]). In none of these cases do the microscopic findings absolutely agree as to the changes present. All are agreed that the monileform condition exists from the very start of the growth of the hair. Ciarocchi found no alterations in the hair follicles at all. Beatty and Scott found a thickening of Huxley's layer of the internal root sheath opposite the constricted parts of the hairs. This we have not been able to confirm, for, as is seen in Plate IX. Fig. 8, Huxley's layer is not any thicker opposite the constrictions than elsewhere. Bering found that each hair was cut transversely at several levels, showing that the hair was twisted up inside the follicle. Guszmann's case was very thoroughly examined, both vertical and horizontal sections being cut. He found the outer root sheath at its inner border, as in our case, slightly sinuous, corresponding to the sinuosities in the outline of the hair, but the layers of the inner root sheath could not be made out at all, being fused into a homogeneous mass. He also found that several follicles united at the level of the sebaceous gland orifice, so that as many as five hairs projected from one follicle. This was not seen in any of our sections.

A very striking point is the regularity with which all the constrictions occur at exactly the same level in each hair. In Plate IX. Figs. 5 and 6, which are from two different follicles, this is well seen. Beatty and Scott also remark

on this formation of nodes or internodes simultaneously all over the scalp. Bering found in his horizontal sections that where he saw four or five hairs together in one follicle, the hairs were not all cut at areas of the same thickness. Therefore he concluded that all the nodes or internodes were not formed synchronously. But that appearance can be explained by the fact that all the hair bulbs are not at the same level in the skin, and therefore all the constrictions are not at the same distance from the surface although all at the same distance from the hair bulbs.

In all our sections the sebaceous glands are very few in number and small in size. This is also in accordance with the findings of Bering and Guszmann, and may account for the dry horny accumulations at the orifice of the follicles thus preventing the free exit of the hair. The poorly developed elastic tissue is also a sign of an atrophic state of the skin. The marked cellular infiltration around the blood vessels is of the nature seen in mild chronic inflammatory processes, and would seem to be probably secondary and possibly due to the irritation produced by the obstruction of the follicles. At any rate it does not seem to have any relation to the growth of the hairs as it does not occur anywhere near the hair papillæ.

Unfortunately these microscopic findings do not give us much assistance as to the causation of this condition. Numerous theories have been brought forward to explain it. Heredity plays an important but, as has been seen, not essential part. The association with keratosis pilaris, which is looked upon by Brocq and others as a localised manifestation of ichthyosis, also points in this direction. Payne blamed Rickets in his case. Francis's case had gland and lung tuberculosis, and in this connection it is noteworthy that one of our cases (William S.) had a patch of lupus vulgaris on the back of his neck.

The three chief theories may be summarised as (1) the parasitic, (2) the mechanical, and (3) the tropho-neurotic.

The *parasitic* theory has practically nothing to support it. The nodes on the hairs in trichorrhexis nodosa are admittedly due to the action of a bacterium, and in piedra and lepothrix to the accumulation of bacteria and fungi respectively, but in monilethrix no one has ever found any signs of bacteria or fungi either on or in the hairs or in the follicles.

The *mechanical* theory has been brought forward by several persons. Bonnet ([14]) found, on examining microscopically the skin of horses during the casting of their coat, the young hairs coiled up upon themselves and showing spindle-like thickenings here and there. These he explained as due to a constriction at the level of the sebaceous gland around which are numerous elastic and longitudinal and circular muscle fibres. These by their contraction cause a narrowing of the follicle, and the soft growing hair has difficulty in getting past this constriction and accumulates as a spindle-shaped mass below. This mass when it reaches a certain size is pushed up past the narrow

area till it slips through, and, once through, the hair grows for a time as a very thin hair which readily becomes coiled up upon itself. Then the constriction by contraction of the muscle fibres becomes too great and another spindle is formed, and so on. In the case of monilethrix, however, the conditions are not similar. There is no constricted area of the follicle through which the hair must pass. There is no muscular tissue and only poorly developed elastic tissue around the areas where the constrictions are produced. Bering and others have tried to explain it by the presence of the keratosis pilaris at the orifice blocking the outlet and causing the hair, which is being pushed up from below, to form spindles. Bering put a thin cylinder of wax in a glass syringe and packed it fairly well round with sand to prevent bending, heated the syringe in a water-bath till the wax was pliable, and then pushed the piston of the syringe slowly in and found that the wax on cooling showed regular spindles. I have repeated this experiment, but failed to get the same results. The wax coiled up upon itself but never showed the least tendency to form spindles. An exactly similar coiling up of the hair, as is well known, occurs in keratosis pilaris, where the coiled-up hair lies in and just below the horny plug at the orifice of the follicle. To the keratosis of the follicle in monilethrix I attribute the coiling up of the hairs as reported by Archambault (3) and others. Therefore the mechanical obstruction at the outlet, while explaining the coiling up, does not explain the spindle formation.

The *tropho-neurotic* theory is the one which seems, so far as our present knowledge goes, to be the most feasible. The alternate thick and thin part of the hair can be explained by assuming an alternate normal and abnormal activity of the cells of the hair bulb. This is the view expressed by Walter Smith, Virchow, Morris, and others. Why the cells should not proliferate regularly is difficult to explain. Thin (48) puts it down to some congenital defect in their structure, but microscopically they look like the cells of normal hair bulbs. Unna locates the fault in the cells which produce the medullary part of the hair, and thinks that they proliferate at regular intervals only. When they proliferate a normally formed part of hair with a medullary canal is produced, but when they cease to proliferate for a time a piece of hair is formed with only a cortex and no medulla, and this cortex becomes compressed together to form the constricted parts of the hair. He also suggests that some vasomotor change at regular intervals in the hair papilla might account for the intermittent growth. Beatty and Scott, Sabouraud and Breda (15) think the intermittent growth is due to some abnormal nerve control of the hair bulb itself. In favour of this nervous theory are the facts that in Unna's case the condition began after a mental shock and in Breda's case, an epileptic, the formation of moniliform hairs coincided with the occurrence of fits. Behrend suggested that the thick parts of the hairs

were the growth during the day, and the constrictions the growth during the night. Ciarocchi found by shaving the head and watching the growth that a spindle and a constriction were formed in each twenty-four hours. It may be that the loss of nerve control during sleep is sufficient to cause a temporary cessation or diminished activity of growth in the cells of the hair bulb.

As no treatment hitherto tried has been known to influence the condition, X-rays were applied to a patch above the left ear for fifteen minutes at a distance of about six inches from the tube. The hair came out almost completely after three weeks and there was a marked disappearance of the keratosis pilaris over that area and an increase in the number of normal hairs (Plate VIII. Fig. 2). Later the whole scalp was X-rayed and now there seem to be more normal hairs than formerly, although the great majority of the hairs do not seem to have been affected. The static breeze was applied seven times at intervals of three days to the top of the scalp, but without result.

With a view to try to alter the circulation in the hair papilla a Clapp's cup was applied to an area near the crown for half an hour daily for nine days, but no alteration was produced in the growth of the hairs.

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DESCRIPTION OF PLATES VIII. AND IX.

Plate VIII.

Fig. 1.—Photograph of scalp before treatment, showing baldness and prominence of follicles.

Fig. 2.—Photograph of scalp four weeks after X-rays, showing round area above and in front of left ear where keratotic plugs in hair follicles have disappeared.

Fig. 3.—*a*, *b*, *c*, *d*—Hairs from cases. (× 400.)

PLATE IX.

FIG. 4.—Section stained with hæmatoxylin and eosin, showing moniliform hair in follicle, absence of sebaceous gland, and presence of cellular infiltration around blood vessels. (× 30.)

FIGS. 5 and 6.—Sections of hair follicles stained by hæmatoxylin and eosin, showing constriction a short distance above bulb. (×70.)

FIG. 7.—Section of mouth of follicle stained by hæmatoxylin and eosin, showing keratotic plug in orifice and cellular infiltration around. (× 70.)

FIG. 8.—High-power view of constriction seen in Fig. 6. (× 600.)

FIG. 9.—Section stained by polychrome methylene-blue, showing cellular infiltration around blood vessels and numerous mast cells stained red. (× 600.)

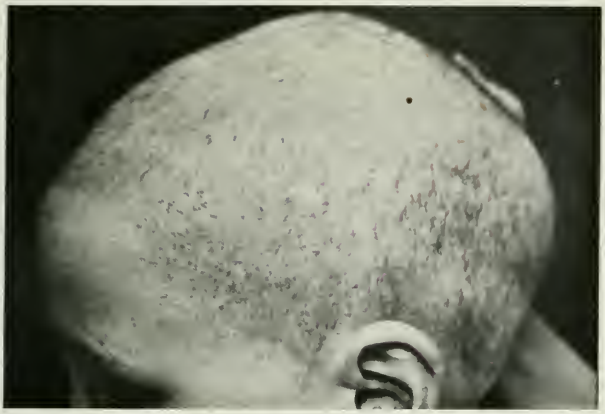


Fig. 1.

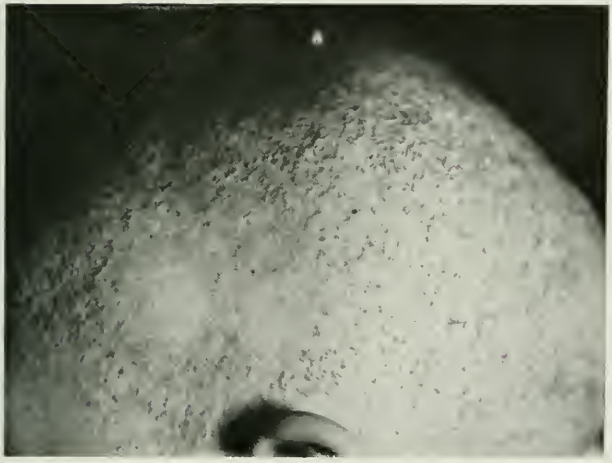


Fig. 2.

Fig. 3

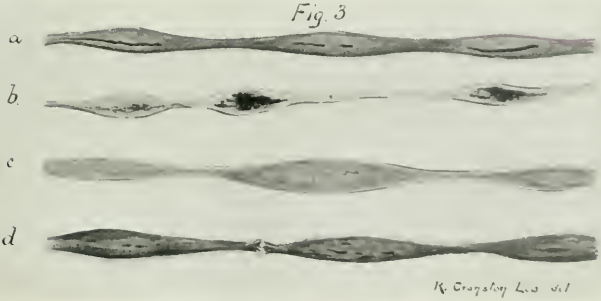


Fig. 3.

Fig 4.

Fig 5

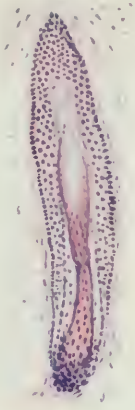


Fig 6

Fig 7

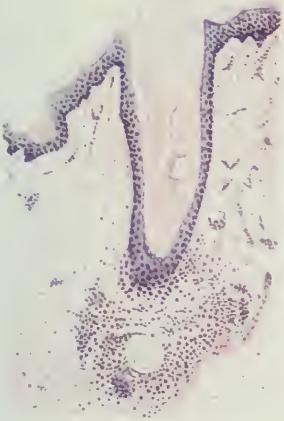
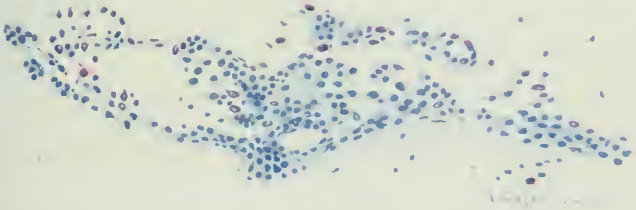


Fig. 8.

Fig 9



ANAPHYLAXIS IN THE RABBIT; THE MECHANISM OF THE SYMPTOMS.[1]

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(Plates VII.–XI.)

Introductory.

In a preliminary communication (an abstract of which appeared in this Journal[2]), it was shown that there were great differences in the severity of the symptoms exhibited by rabbits "sensitised" by a previous injection of foreign serum on re-injection with the same. Those differences corresponded with differences in the amount of precipitin produced in response to the first injection. With abundant precipitin the symptoms were severe and the result often fatal; with precipitin less marked the symptoms were mild and were followed by perfect recovery. During the anaphylactic illness it was shown that a diminution of the animal's serum-complement occurs, *i.e.* a larger dose of the fresh serum is required than before to dissolve the same combination of washed blood corpuscles and hæmolytic immune body.

The present paper discusses the mechanism of the symptoms in the rabbit.

I. Conditions of Production of the Anaphylactic Illness.

The illness referred to is that following immediately upon intravenous injection; the phenomena obtained by other routes of administration—subcutaneous, intraperitoneal, or intracerebral—are not included, nor are such late effects as death after twenty-four hours.

This immediate illness is remarkably uniform and does not vary with the different proteins, but has been produced in my experiments with substances of such diverse origin as serum (equine, bovine, and human), milk, foreign blood corpuscles (human and bovine), bacteria, and the vegetable protein of the rice

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[2] *Journ. Path. and Bacteriol.*, Cambridge, 1910, vol. xiv. p. 117.

seed. These substances may be given to fresh rabbits in relatively large amounts, *i.e.* there is little original toxicity. The first or preparatory injection may be either subcutaneous, intraperitoneal, or intravenous; the latter seems to give the best results.

The dose of protein should correspond to about 5 c.c. of blood serum per kilo body weight; larger doses are certainly of no advantage. The low limit of dose for producing the sensitive condition probably lies about 1 c.c.

It is important to note that the minute dose which it is essential to use in sensitising guinea-pigs towards foreign serum has no effect in the rabbit. Of six fresh rabbits which received each 0·05 c.c. horse serum intravenously, not one showed symptoms on a subsequent injection.

The repeated administration of rabbit serum to rabbits fails to produce any but slight and doubtful disturbance. An interval of ten days must elapse from the first injection for the sensitive condition to develop; it then remains of the same degree for other ten days, thereafter rapidly diminishing. This is again in contrast to the guinea-pig, which remains highly sensitive for prolonged periods.

An intravenous dose, in the case of serum say 3 c.c. in such a prepared animal is followed by the symptoms to be described. Should the animal recover, repetition of the same or a much larger dose produces not the least disturbance. The condition of "anti-anaphylaxis" or "refractoriness" has appeared. This persists for three or four days: on the fifth day the sensitive condition reappears, to be in full force on the seventh. There is an acceleration thus in the rate at which the sensitisation occurs after the second and subsequent doses as compared with the first.

TABLE I.

Description.	Time since last Injection.	Precipitin.	Dose Serum.	Symptom.
(1) Russian, ♀ ; 2600 grms.	11 days.	Strong.	3 c.c.	Severe: death in four minutes.
(2) Dutch, ♀ ; 1550 grms.	11 ,,	Trace.	3½ ,,	None.
(3) White, ♂ ; 1720 grms.	10 ,,	Strong.	2½ ,,	Severe: recovered in fifty minutes.
(4) Russian, ♀ ; 1670 grms.	10 ,,	Very weak.	2½ ,,	Slight: recovered in ten minutes.
(5) Dutch, ♀ ; same individual as No. 2.	18 ,,	Very weak.	1 ,,	Doubtful.
(6) White, ♂ ; same individual as No. 3.	20 ,,	Strong.	2½ ,,	Severe: recovered in one hour.
(7) Russian, ♀ ; same individual as No. 4.	20	Very weak.	1 ,,	Slight: recovered in six minutes.
(8) Grey Lop, ♂ ; 2800 grms.	20 ,,	Weak.	1 ,,	Moderate: recovered in fifteen minutes.
(9) Grey Lop, ♂ ; same as No. 8.	8 ,,	Strong.	3 ,,	Severe: died in seven minutes.
(10) Black, ♀.	12 ,,	Moderate.	3 ,,	Severe: recovered in twenty minutes.

Passive transference of the sensitive condition can be effected. A dose of 5 or 10 c.c. of the serum of a sensitive rabbit immediately confers on a fresh rabbit the capacity to react with the typical symptoms on injection with the appropriate protein. As a rule, however, the symptoms are distinctly milder; they are certainly more transient. These conditions are markedly different from the case of the guinea-pig where the serum of the sensitive guinea-pig must be given at least twenty-four hours before the assaulting dose. In the rabbit this procedure produces symptoms much less definite than when the two injections are given practically simultaneously.

There are very marked variations in the degree to which individual rabbits can be sensitised, *i.e.* in the degree of severity of the symptoms that may be induced by a second or subsequent intravenous injection. Of thirty rabbits prepared in much the same way, five succumbed to a second dose of from 2 to 5 c.c. of the foreign serum, other six died acutely as a result of subsequent similar doses; the remainder, though some were frequently rendered severely ill, never developed a fatal degree of susceptibility. Among these also, the severity of the symptoms on injection varied on different occasions in the same rabbit. The reason for the different aptitude for sensitisation in different rabbits is not known. Neither age, sex, race, nor feeding could be observed to have any relation to it. It goes, however, hand in hand, as has been mentioned, with the degree to which, in serum anaphylaxis, the animal develops precipitin. Table I. illustrates this; it represents the clinical behaviour, along with the degree of precipitin present at the moment of anaphylactic assault and the history, in the case of ten experiments taken simply in the order found in the course of my notes. The method of precipitin estimation employed will be described later.

II. The Symptoms Observed in Anaphylactic Rabbits.

In a rabbit with strong precipitin in its serum the intravenous injection of 3 c.c. of the appropriate foreign serum sets up the following symptoms.

Within fifteen seconds of starting the injection—in fact, usually before it is completed—there is a well-marked blush of the ears, the veins swelling up and even pulsating; this lasts for a period of from a second or two to about a minute, and is associated with alteration in the rhythm of the breathing,—little pauses and shortened breaths. Frequently at this stage there is some restlessness, the animal pricks up its ears and hops about. Following sharply on this fleeting hyperæmia comes pronounced anæmia of the ear, the veins collapse, the ear becomes cold, and strong heat and friction fail to produce the normal reaction of congestion; at this stage it is impossible to bleed; the ear vein cut across yields not a drop of blood, and even the technique of bleeding with an incandescent electric bulb to induce continuous congestion fails entirely.

Along with this there is marked panting respiration, differing from the normal panting of the rabbit by its greater vigour and depth; the heart beats more rapidly and feebly, becoming almost impossible to count. The animal is restless, seeking apparently a comfortable attitude and holding its head high with an anxious expression.

At the second or third minute muscular weakness appears; the animal lies prostrate with legs stretched out and head resting on the floor; the breathing is heaving and laboured, the heart beat rapid and feeble; no notice is taken of sounds and vigorous disturbance; shaking and flicking elicits little or no response; the eyes are half shut, and the expression is one of deep sleep. The abdomen at this period presents a striking appearance; it is large

and full, but the heaving movements of the diaphragm and chest transmit little or no excursion to it; the outlines of the stomach and cæcum show through, and loose folds of the flaccid muscular wall seem to flow out from the side of the prostrate rabbit. The skeletal muscles generally have lost tone; the spine bends, and the limbs hang limply when the body is lifted. Fæcal matter is discharged, first solid, then loose and liquid; if the bladder happens to be full, urine is also discharged.

It is still practically impossible to get blood from the ear; any drop that may be expressed is very dark and tarry, and coagulates slowly.

In five to ten minutes this stage of depression reaches a maximum, and rapid death may occur. Lying on its side, the rabbit begins to kick convulsively; the breathing ceases, to be replaced by loud intermittent gasps; the head is thrown back, with nostrils wide and eyeballs protruding. There may be two or three of these convulsive seizures at intervals of a few seconds, with severe spasms of the back muscles and gasping or screaming attempts at respiration; then the respiration ceases, the corneal reflex disappears, the animal is dead. The heart usually continues to beat for several minutes, then gradually slows and stops. In non-fatal cases all degrees of severity may be observed, from those where the only evidence of disturbance may be a transient panting and local anæmia of the ears, with complete recovery in five to ten minutes, to those where for thirty minutes the animal is in a state of deep coma, recovering in the course of the following two hours.

The condition in human nosology which most nearly resembles the anaphylactic illness is that of surgical shock; there is as in this a low blood pressure with stagnation in the portal area on which adrenalin has little effect; there is extreme muscular weakness, shallow breathing, and a condition approaching unconsciousness; recovery, as in shock, may be rapid and complete.

III. The Mechanism of the Symptoms.

In a kymograph tracing of an animal subjected to anaphylactic assault with a moderate amount of precipitin in its serum, *i.e.* an animal likely to have had clinically mild symptoms, a fall of arterial blood pressure commences within half a minute of starting the injection. It progresses by slow degrees, reaching its lowest point in from seven to fifteen minutes. Thereafter it slowly rises, and during the succeeding ten minutes very nearly reaches its original level. (Plates VIII. and IX. Figs. 7, 8, 9, and 10.)

In a severe or fatal case the maximum depression is reached more rapidly. In three or four minutes the blood pressure may fall from 100 to 30 mm. of mercury. The pulse waves are more rapid, but smaller in size. The respiration is markedly accelerated, and the excursions of the chest wall greater. The fatal issue is signalled by the sudden stop of the respiration—usually from four to ten minutes from the start.

The blood pressure rises slightly (probably an asphyxial rise), then falls to base level in the course of three to four minutes, the heart continuing to beat steadily long after the respiration has ceased. Spasmodic respiratory movements occasionally appear at this stage without much effect on the blood pressure curve. (Plates IX., X., and XI. Figs. 11, 12, 13, 14, 15.)

The immediate explanation of the symptoms, the prostration, and muscular paresis is apparent: the profound fall of blood pressure

accounts for all. The respiratory arrest probably demands another explanation; to it the agonal convulsions are to be ascribed.

IV. The Cause of the Fall of Blood Pressure.

The mechanism of the fall of blood pressure is less obvious. Paralysis of the muscular wall of the arterioles, as in nitrite poisoning, produces a profound fall of pressure. In the tracing, however, the waves of the heart beat tend to increase in size as the pressure falls, while the whole effect can be antagonised by adrenalin. But in the anaphylactic depression the curve resembles more that obtained when an animal is rapidly exsanguined. The heart waves become smaller as the pressure falls, and adrenalin produces merely an evanescent rise of pressure without ameliorating the symptoms.

Further, a striking point in the symptomatology is the swelling of the abdomen which persists throughout the illness. This, as is seen post-mortem, is due to an intense engorgement of the portal system of vessels.

Arteriole paralysis alone cannot account for this condition: such a paralysis would, if the total volume of the capillary channels remained approximately unaltered, rather tend to relieve portal stagnation by transmitting unchecked the wave of increased pressure from the heart beat so as to aid the venous flow. In nitrite poisoning, capillary distension in the abdomen is relatively mild, producing, in spite of the arteriole paralysis, no such intense portal congestion as in the anaphylactic rabbit.

These differences can only be explained by supposing in anaphylaxis some intense dilating action on the capillary vessels themselves, as a result of which the systemic circulation is rapidly depleted into the portal, and reduced to the same condition as after a severe hæmorrhage.

Such an intense dilating action can be produced either (*a*) by a sudden mechanical obstruction to the blood flow in the inferior vena cava in the thorax, as has been shown by Bolton (1909 [3]), *i.e.* by a passive distension by backward pressure; or (*b*) by primary dilatation of the capillary vessels themselves.

Paralysis of the capillaries as an independent possibility has had little attention paid to it by pharmacologists, although Roy and Graham Brown (1879 [8]) showed that the capillaries of the frog possessed an independent contractility, and that their lumen was not affected by variations of the blood pressure within wide limits. More recently the same has been shown by Steinach and Kahn (1903 [10]) in the mammal,—a variation in the diameter of the capillaries independent of blood pressure and directly presided over by the sympathetic system.

There are again two possibilities: the widespread relaxation of the tonus of the capillary vessels may be the result of sympathetic nerve

activity (or its removal), or it may be due to direct injury to the endothelial cells which build them up.

V. Histological Appearances.

The histological appearances are of importance in this connection.

In the organs of the splanchnic area the general congestion which is apparent naked-eye, not only in those dying after respiratory arrest, but in those bled to death during the height of the symptoms, is shown microscopically by an intense capillary engorgement with minute hæmorrhages. In the intestinal villi in particular the capillaries are represented by large blood spaces with a lining of thinned-out endothelium; the submucous tissue shows distended venules with here and there rupture and extravasation. (Plate VII. Figs. 1 and 2.) Such capillary hæmorrhages, it is to be noted, do not appear in nitrite poisoning.

The intralobular capillaries of the liver are dilated and full of blood; both the central veins of the lobules and the twigs of the portal vein in the portal spaces are similarly distended. The omentum, spleen, and pancreas are equally congested; the stomach often shows but slight changes. The lungs retract normally on opening the thorax, and are rather pale; microscopically there is a characteristic thickening of the interalveolar septa; the capillaries are compressed, and the contained blood corpuscles seem to be peculiarly adherent to the walls.

Some alveoli contain a little serous exudate; there is no emphysema as in the guinea-pig, and never a general œdema. The lung condition, however, certainly suggests a very early stage of an acute œdema. (Plate VII. Figs. 3, 4, 5, and 6.)

VI. Blood Changes.

The blood changes next require attention. During the anaphylactic illness, as has been said, the blood is very dark in colour and slow to coagulate.

(*A*) *Delayed Coagulation.*

Instead of furnishing a thread of fibrin (McGowan's capillary tube method) in six or seven minutes as before injection, the blood sets to a jelly only after ten to fifteen minutes; the formation of a fibrin thread is usually very indefinite. The striking change is that the serum almost always fails to separate spontaneously although the clot is firm. The phenomenon never approaches the marked condition of the peptone plasma in the dog, where the blood remains fluid for days. The abnormal factor would seem to be primarily a loss of the property of retraction rather than a marked deficiency in fibrin-ferment or fibrinogen.

(*B*) *Leucopenia.*

From 10,000, say, the leucocytes fall practically immediately to 3000 or 1000 per cm. Differential counts show that it is chiefly the polymorphs that have disappeared; those remaining are almost all mononuclear, and display a marked tendency to clump. The leucopenia, however, is the least uniform of the blood changes. Occasionally no fall in the count appears. (The complement is none the less diminished in these cases, which negatives a causal connection of the leucopenia for the latter.)

(*C*) *Precipitin.*

The precipitin, it is important to note (in serum anaphylaxis), completely disappears. (It reappears, in animals which recover, in three to four days, its reappearance coinciding with the regaining of susceptibility.)

(*D*) *Destruction of Complement.*

The complement is found to be markedly weaker than before injection. The minimum hæmolytic dose before, having been, say, 0·15 c.c., is now 0·7 to 0·8 c.c., or the serum may possess practically no hæmolytic power whatever. In the slighter degrees of illness the complement is proportionally less affected. As the animal recovers the complement strength returns to its former height—usually within one or two hours, even after severe symptoms.

(*E*) *The Reactions of Natural Immunity.*

It is of interest to note that it is not merely the hæmolytic property of the serum, unimportant under natural conditions, but the defensive bacteriolytic also that is affected. The fresh serum obtained before the anaphylactic illness is capable of sterilising itself at 37° C. after the addition of as many as 100,000 living vibrios per c.c.; the serum obtained during the illness is rapidly overgrown with the vibrios after so small an addition as some tens of vibrios only per c.c., *i.e.* fails to protect itself against the slightest invasion. (The method employed for these results was that of Wright (1905 [11]), which is peculiarly suitable for comparative tests with fresh serum.)

The opsonic property is similarly thrown out of action under those conditions. Both for tubercle bacilli and for staphylococci the opsonin almost disappears. The count per cell may drop from 10 with the serum before injection to 0·5 after. The mean of twenty different experiments, in most of which the illness was relatively slight, gives a figure of 6·7 per cell before injection, 1·9 after injection. The effect of such changes on the resistance to infection can hardly be questioned.

In Table II. representative examples of the blood changes which occur during the anaphylactic illness are put together.

Table II.

Period.	Precipitin.	Coagulation Time.	Leucocytes.	Hæmolytic Complement.	Bacteriolytic Power.	Opsonin.
Immediately before injection: ten days since last dose.	Strong.	Fibrin thread at six minutes.	10,000 per cm.	0·15 c.c. = M.C.D.	120 vibrios per cm.	6·5 bacteria per polymorph.
Five minutes after injection.	None.	Jelly formation about fifteen minutes.	3,000 per cm.	0·6 c.c. = M.C.D.	Nil.	2·0 bacteria per polymorph.
Two hours after injection.	None.	Fibrin thread at six minutes.	12,000 per cm.	0·15 c.c. = M.C.D.	–	–

Methods.

(*a*) The precipitin estimation was conducted in capillary tubes of 3 mm. diameter drawn to a point at the closed end. Antigen, *e.g.* horse serum, was diluted with 0·85 per cent. saline to six different strengths—1 : 10, 1 : 100, 1 : 500, 1 : 1000, 1 : 5000, 1 : 10,000. Of each of these 0·2 c.c. was filled into capillary tubes with a fine pipette, beginning with dilution 1 : 10,000. With a fresh pipette 0·2 c.c. of the rabbit serum to be tested was then allowed to run down the side of each, displacing the diluted antigen and giving a zone of mixture of about 2 mm. In this zone turbidity appears and flocculation in proportion to the amount of precipitin present. (This procedure is better than the usual layering method for comparative tests between two sera.) From the height of the dilution still producing turbidity, and from the degree of turbidity and amount of deposit in twenty-four hours, the sera were classed as "very strong," "strong," "moderate," "weak," "very weak," or "trace precipitin." In practice no more accurate method is available; accurate measurement of the precipitate as in Nuttall's method would necessitate first working out for each serum the optimum dilution for precipitation—this varies considerably in different sera—before correct comparisons as to the amount of precipitable antibody could be instituted.

(*b*) The complement estimation was conducted in small test tubes of about 4 c.c. capacity carefully cleaned before use. The fresh serum to be tested was first added in decreasing amounts, then two minimum hæmolytic doses of rabbit "I.B." *v.* ox corpuscles; then saline to bring the contents to 1 c.c. in all the tubes, after which 1 c.c. of a 1 per cent. suspension of freshly washed—not more than two days old—ox blood corpuscles was rapidly mixed with each, and the whole put at 37° C. The degree of lysis was read after half an hour, after two hours (in the incubator), then after twenty hours at room temperature.

VII. The Connection with the Sympathetic System.

What connection is there between these blood changes and the general disturbance?

Phenomena remarkably resembling these are seen on section of the abdominal sympathetic nerve trunks. Rüchel and Spitta (1903[3]) cut the splanchnics in rabbits and found a profound fall of blood pressure, portal congestion, immediate leucopenia, and delayed blood coagulation—in a word, some of the cardinal phenomena of the anaphylactic syndrome. The interdependence of these peculiar changes has been explained thus. The loss of the nervous impulses produces a widespread loss of tone in the vascular tissue formerly supplied, both arterioles and capillaries; in consequence a pronounced stagnation of blood results. The systemic circulation is rapidly depleted into the portal, and the blood pressure falls; the leucocytes, in particular the more responsive polymorphs, settle quickly out of the slowly circulating blood—especially perhaps in the liver—leaving the systemic circulation relatively poor. The weak retracting power of a blood clot poor in leucocytes is a well-known observation.

An explanation such as this may have some bearing on certain of the blood changes of the anaphylactic syndrome. It is not evidence, however, that there actually is disturbance of the sympathetic nervous system as a result of the anaphylactic assault. It is difficult to suppose, besides, that the sympathetic alone of the

nervous system should be so profoundly affected. In one of my kymograph experiments, where the abdomen was opened and a marked fall of blood pressure produced by handling the intestine, *i.e.* by sympathetic shock, the injection of the assaulting dose nevertheless produced an equally definite further fall, indicating at least a more powerful action on the part of the latter than sympathetic shock alone was capable of producing.

VIII. Injury to the Capillary Endothelium.

By a process of exclusion thus we are forced to postulate an injury to the capillary walls themselves for the production of the anaphylactic effects.

Objective evidence of this is not easy to furnish. Histological methods of greater delicacy than have hitherto been applied are required to bring to the eye such changes in the cells concerned,—which cannot, after all, considering the rapidity of recovery, be of a gross character. The prevalence of capillary hæmorrhages is of course suggestive.

Consideration of the effects of subcutaneous injection of the assaulting dose (Arthus and Breton, 1903[1]) tends in the same direction. Here no general disturbance occurs, the whole violence of the reaction being spent at the site of inoculation, and producing a widespread acute hæmorrhagic œdema—the phenomenon of Arthus. The probability is apparent that the same process, occurring within the circulating blood, should profoundly affect in a similar manner the general vascular endothelium—the cells in most direct proximity.

Direct injury to the capillary endothelium as the explanation for the pharmacological action of certain poisons has been strongly maintained by Heubner (1907[7]). A group of substances chemically widely different exists, characterised by a typical syndrome on intravenous injection. Among these are tartar emetic, double salts of iron, nickel, gold and platinum, and the alkaloid emetine.

In the rabbit this common syndrome consists in a rapid fall of blood pressure and portal congestion of intense degree with capillary hæmorrhages, death resulting by respiratory arrest, the heart continuing to beat for some time. Delayed blood coagulation and leucopenia are strongly marked. The points of similarity to the anaphylactic illness are apparent.

IX. The Lung Condition.

The cause of the sudden respiratory arrest is still to be explained. The supposition that the low blood pressure, causing a profound anæmia of the respiratory centre, is responsible, is rendered improbable by the fact that artificial respiration after the breathing has ceased does not prolong life: the heart stops beating with marked engorgement of the right chambers in spite of it. The manner of the death,

too, with gasping, convulsive attempts at respiration, suggests strongly that it is of a suffocative character.

There is no evidence, however, in the rabbit of the bronchiole spasm with acute emphysema which causes the rapid anaphylactic death in the guinea-pig (Auer and Lewis, 1910 [2]). The histological appearance described rather indicates a commencing œdema of the lung, insufficient to produce mechanical respiratory arrest. Further investigation of this point is necessary.

X. The Cause of Anaphylaxis.

What modification has taken place in the economy of the animal to lead to behaviour so vastly different from the normal? The fact of passive transference of the sensitive condition by means of the serum fixes attention on this as containing the responsible factor. Further, the remarkable correspondence already described between the precipitin content of the serum and the severity of the symptoms at once suggests that the presence of this, and not of any other hypothetical anaphylactic antibody, is the new factor to which is due the difference between the normal and the sensitive animal. Strong proof of this has been furnished by Doerr and Russ (1910 [4]). These observers, employing the normal guinea-pig as test animal, estimated the power of different samples of sensitive rabbit's serum to produce passive anaphylaxis, and worked out the minimum dose of this that—plus a standard dose of antigen—still produced death. The precipitin strength of the sera was found in exact proportion to their lethality. Further, the precipitate itself obtained *in vitro* by separation from a mixture of precipitin rabbit serum and antigen is definitely toxic, as shown by the same authors.[1] The disappearance of the precipitin from the serum after the illness and the "refractory" condition during its absence are also points of presumptive evidence.

How the interaction of precipitin and antigen acts so as to produce the morbid changes described is still a matter for speculation.

The conditions in the anaphylaxis to foreign blood corpuscles are little clearer; here the hæmolytic immune body is precisely in the position of the precipitin in serum anaphylaxis.

Friedemann (1909 [6]) has reproduced the anaphylactic illness by mixing hæmolytic immune body, fresh complement, and washed blood corpuscles *in vitro*, removing the latter, before lysis is advanced, with the centrifuge, and injecting intravenously the supernatant, now toxic, fluid. This, then, would represent the true poison, *i.e.* an early product of cytolysis, which is of course a process much more rapid and complete in the treated, "immunised" animal than in the normal. Friedemann describes a fall of complement in the cyto-anaphylactic animal which I can confirm and which completes the similarity to the serum anaphylaxis. Friedberger (1910 [5]) has recently published an important experiment in imitation of that of Friedemann above mentioned.

[1] *Loc. cit.*

He has been able to extract, by means of fresh guinea-pig serum ("complement"), a toxic substance from the precipitate of rabbit precipitin plus antigen, with which in guinea-pigs the typical anaphylactic death can be produced. This "anaphylotoxin" cannot be extracted by normal saline, but requires the complement. This substance it is which is the final stage in the interaction of antigen and antibody in the circulation, and to which alone the disturbances are due.

XI. The Significance of the Fall of Complement.

Friedberger (1910 [5]), on the strength of the experiment just quoted, considers that the antigen-precipitin combination is merely a potentially toxic substance, requiring for the liberation of the actual toxin, the "anaphylotoxin," the intervention of the complement. Thus is to be explained the diminution in complement in the anaphylactic illness; it has been used up in the preparation of the "anaphylotoxin." Further proof of this is found in the fact that a preparatory injection of hypertonic sodium-chloride solution prevents in the guinea-pig the production of the illness by serum precipitin. This procedure *in vitro*, *i.e.* the addition of strong sodium-chloride solution, is known to inactivate complement; the liberation of toxin, according to Friedberger, is thus prevented.

These experiments, however, are capable of quite different interpretations, and the theory, in my opinion, is not so well founded as to bear the far-reaching conclusions that have been based upon it. Experiments on these points are in progress. The possibility cannot be lightly dismissed that the change in the physico-chemical condition of the blood which makes itself apparent as a weakening of complement may be itself the agent in the "toxic action."

XII. Disturbance of Metabolism.

The rectal temperature after an anaphylactic illness of definite severity is subnormal. The average fall, excluding cases where recovery was complete in fifteen minutes, is between 1° and 3° F. There is no such prolonged hypothermia as occurs so typically in the guinea-pig; the temperature in the rabbit regains the original level rapidly.

To be correlated with this behaviour is that of the respiratory exchange.

Method.

The experiments on this point were conducted with an apparatus constructed by Dr. T. S. M'Intosh, the description of which is shortly to be published. It consists of a glass respiratory chamber through which air is drawn at a measured rate. Samples of the outgoing air are taken at a uniform rate over a definite period,—usually about 10 c.c. of breathed air was collected during thirty minutes,—the ventilation being at the rate of about 150 litres per hour. The samples are analysed in Haldane's gas analysis apparatus. With this apparatus, after applying certain needful corrections,

concordant results are obtained, and it presents distinct conveniences as compared with the weighing method.

TABLE III.

Description.	Symptoms.	Period respired Air collected.	Per Kilo. per Hour. CO_2 exhaled.	Per Kilo. per Hour. Oxygen absorbed.
(1) Black, ♀; young, 900 grms., fasting 20 hours. Passively sensitised; 9 c.c. strong precipitin serum v. horse intravenous.	No symptoms.	¼–¾ hour after injection.	990 c.c.	1106 c.c.
1 hour afterwards 5 c.c. horse serum intravenous.	Marked prostration; recovered in 10 minutes.	¼–1¼ hour after injection.	642 c.c. (=35 p.c. fall).	940 c.c.
(2) Black, ♀; young, 950 grms., fasting 20 hours (control). 9 c.c. normal rabbit serum intravenous.	No symptoms.	½–1 hour after injection.	1092 c.c.	1107 c.c.
1 hour after 5 c.c. horse serum intravenous.	No symptoms.	½–1 hour after injection.	1008 c.c. (fall negligible).	1093 c.c.

TABLE IV.

Description.	Precipitin and Symptoms.	Period respired Air collected.	Per Kilo. per Hour. CO_2 exhaled.	Per Kilo. per Hour. Oxygen absorbed.
(1) Grey, ♂; 1370 grms., fasting 24 hours.	Moderate precipitin.	½ hour before injection.	753 c.c.	823 c.c.
Received 2 c.c. horse serum intravenous.	Considerable prostration.	½–1 hour after injection.	575 c.c.	672 c.c.
...	Recovered; somewhat languid.	2½–3 hours after injection.	559 c.c. (=26 p.c. reduction).	687 c.c.
4 hours after received 5 c.c. horse serum intravenous.	No symptoms, "refractory."	½ hour after "refractory" injection.	701 c.c.	905 c.c.
(2) Russian, ♀; 2580 grms., fasting 20 hours.	Very weak precipitin.	½ hour before injection.	726 c.c.	801 c.c.
Received 2 c.c. horse serum intravenous.	Very slight symptoms.	½–1 hour after injection.	702 c.c. (=negligible fall).	755 c.c.

In Table III. the effects on respiratory exchange of a passively induced anaphylactic shock are to be seen along with the control. The carbon dioxide production has fallen 35 per cent. (The error of experiment was found to work out about 5 per cent.) In Table IV. the effects of a moderately severe "active" anaphylaxis are expressed; with the negative results where the symptoms were slight. In seven experiments, in most of which the symptoms were relatively mild, the diminution in CO_2 excretion averaged 25 per cent.

Whether this disturbance is dependent on the lung condition described or on some general interference with tissue metabolism is not settled.

In conclusion, I must express my deep indebtedness to Dr. Ritchie, Superintendent of the Royal College of Physicians Laboratory, for continued help and invaluable counsel during the progress of the work.

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DESCRIPTION OF PLATES VII.–XI.

Plate VII.

Fig. 1.—Small intestine of anaphylactic rabbit killed by 5 c.c. human serum, intravenous; intense congestion of capillaries in the mucous membrane. (×200.)

Fig. 2.—Small intestine of anaphylactic rabbit killed by 5 c.c. horse serum, intravenous; villus occupied by a large blood space, distended capillary or venule. (×200.)

Fig. 3.—Lung of normal rabbit; general arrangement with thin-walled alveoli. (×50.)

Fig. 4.—The same; narrow alveolar septa. (×200.)

Fig. 5.—Lung of anaphylactic rabbit (the animal of Fig. 2); appearance suggesting collapse. (×50.)

Fig. 6.—The same; thickening of alveolar septa, with crumpling and encroachment on the air spaces. (×200.)

Plate VIII.

Fig. 7.—Kymograph tracing of anaphylactic illness of moderate severity. Urethane anæsthesia (3 grms. subcutaneously), rabbit 2100 grms. (its serum contained a moderate amount of precipitin *v.* ox serum).

Carotid blood pressure before injection = 92 mm. mercury; half a minute after = 84 mm.
Respiration before injection = 78 per minute; half a minute after = 84 per mm.

FIG. 8.—Continuation of above: five to six minutes after injection.
Carotid blood pressure = 68 mm. mercury.
Respiration = 76 per minute.

PLATE IX.

FIG. 9.—Continuation: twelve minutes after injection.
Carotid blood pressure = 44 mm. mercury.
Respiration = 70 per minute.

FIG. 10.—Continuation of above: twenty minutes after injection.
Carotid blood pressure = 72 mm. mercury.
Respiration = 65 per minute (gradual recovery).

FIG. 11.—Kymograph tracing of fatal anaphylactic illness. Rabbit 2300 grms. with strong precipitin serum *v.* horse, urethane anæsthesia; 3½ grms. subcutaneously. (Abdomen opened and intestine handled before tracing commenced; some degree of shock present, with blood pressure lower than normal.) Respiration tracing with chest tambour.
Carotid blood pressure before injection = 72 mm. mercury.
Respiration before injection = 64 per minute.

PLATE X.

FIG. 12.—Direct continuation of Fig. 11.
Carotid blood pressure at end of injection = 46 mm. mercury.
Respiration at end of injection = 114 per minute (= 50 seconds from commencing injection).
Rapid fall of blood pressure, with increased rate of respiration.

FIG. 13.—Direct continuation of Fig. 12 = half a minute after injection.
Respiration suddenly stops: carotid blood pressure rises to 74 mm. mercury.
One and a half minutes after injection gasping attempts at respiration occur; blood pressure fallen to 57 mm. mercury.

PLATE XI.

FIG. 14.—Direct continuation of Fig. 13.
Gasping respiratory movements continue, one every three seconds.
At two minutes after injection, carotid blood pressure = 56 mm. mercury.
At two and a half minutes after injection carotid blood pressure = 36 mm. mercury.

FIG. 15.—Direct continuation of Fig. 14.
Blood pressure falls slowly.
At three minutes after injection carotid blood pressure = 22 mm. mercury.
At three and a half minutes after injection carotid blood pressure = 18 mm. mercury.
Gasping respiration ceased at four minutes. Heart ceased to beat at five minutes after injection.

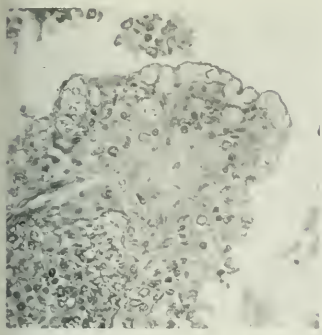


Fig. 1.

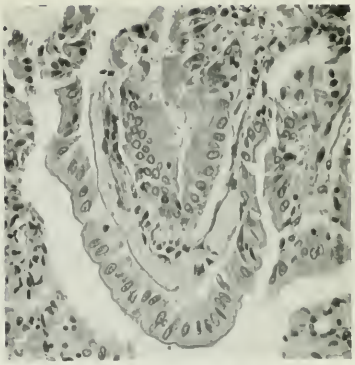


Fig. 2.

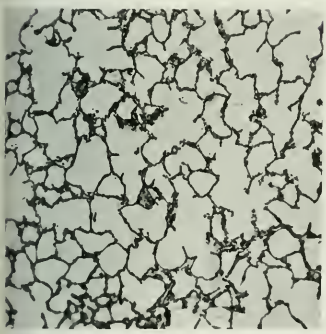


Fig. 3.

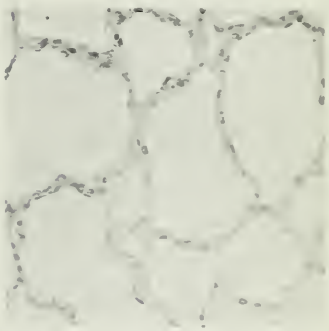


Fig. 4.

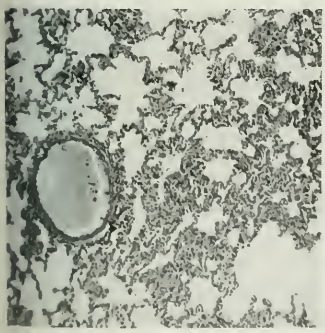


Fig. 5.

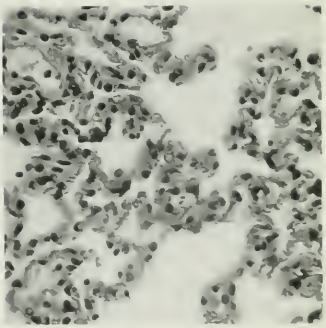


Fig. 6.

FIG. 7.

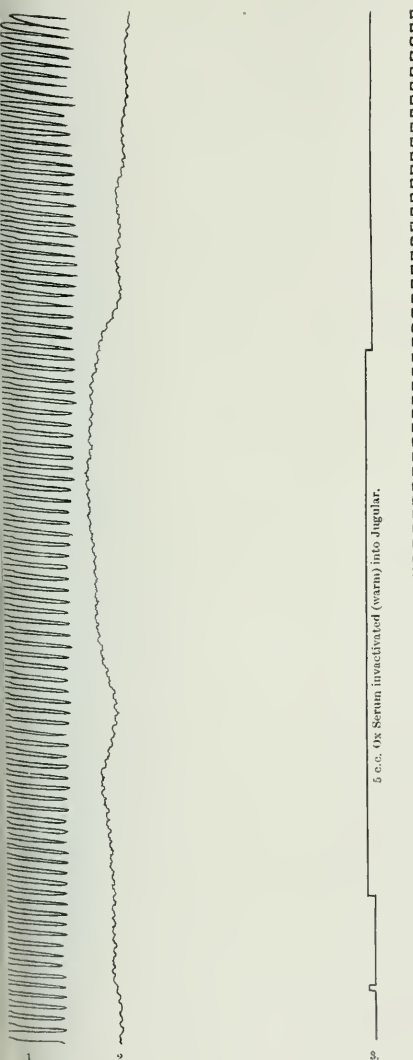
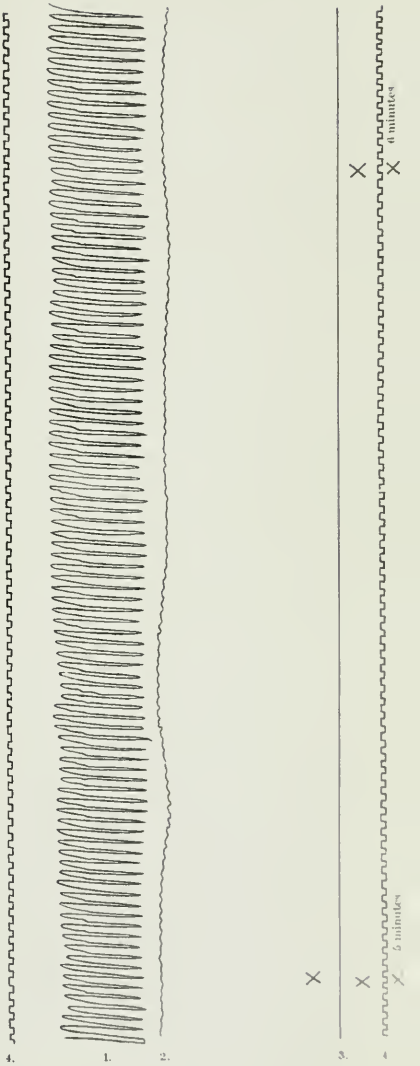


FIG. 8.



1. Respiratory Tracing. 2. Carotid Blood Pressure.
3. Signal and Manometer Abscissa. 4. Seconds.

FIG. 10.

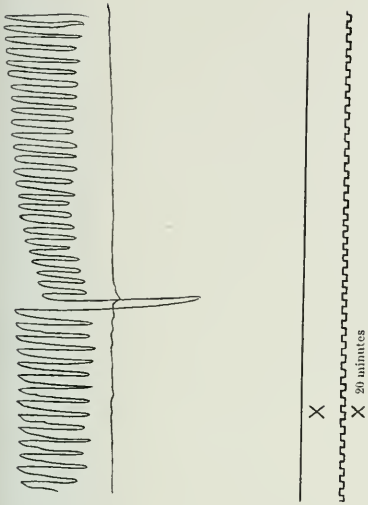


FIG. 9.

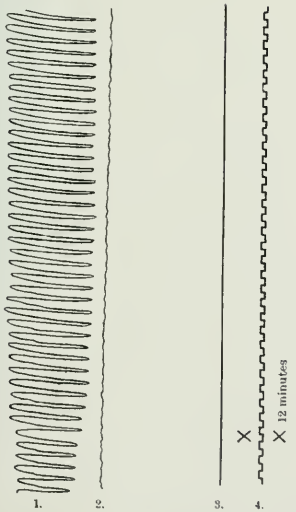
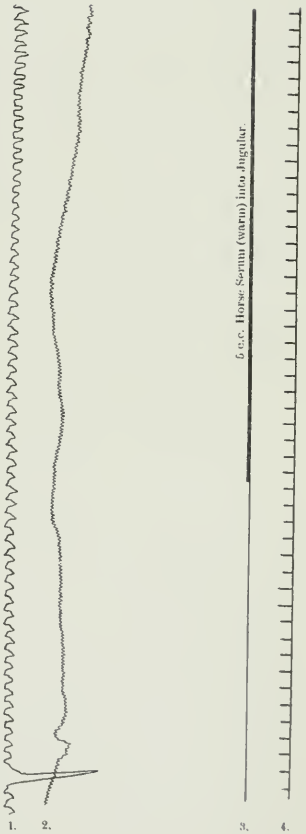


FIG. 11.



1. Respiratory Tracing. 2. Carotid Blood Pressure.
3. Abscissa. 4. Seconds.

FIG. 12.

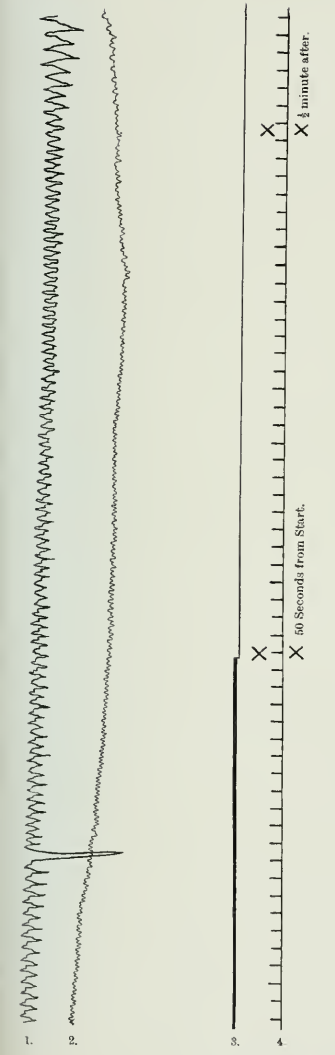
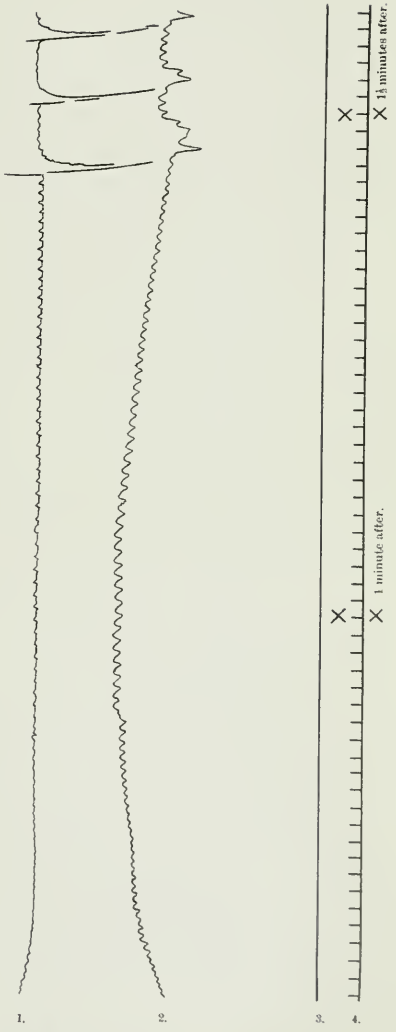


FIG. 13.



1. Respiratory Tracing. 2. Carotid Blood Pressure.
3. Signal and Abscissa. 4 Seconds.

FIG. 14.

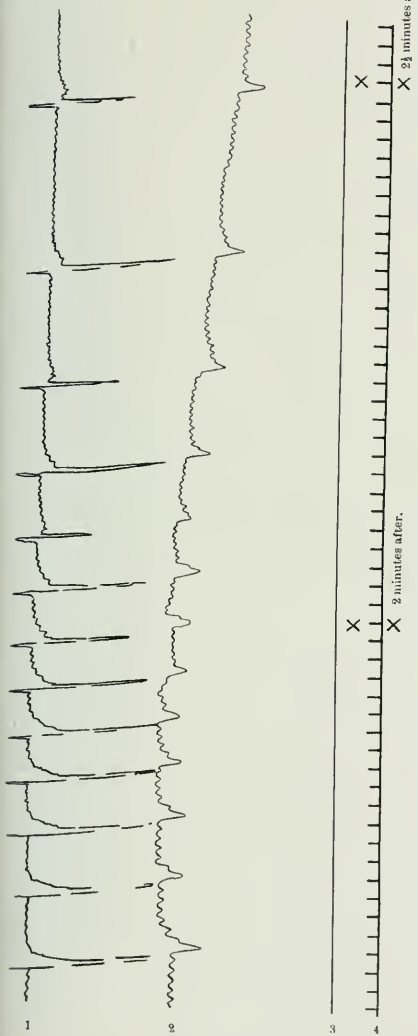
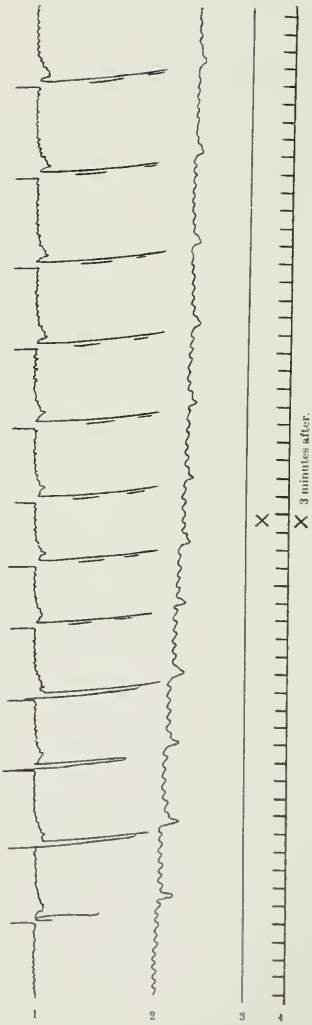


FIG. 15



1. Respiratory Tracing. 2. Carotid Blood Pressure.

3. Abscissa. 4. Seconds.

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XANTHOMA TUBEROSUM MULTIPLEX, WITH LESIONS IN THE HEART AND TENDON-SHEATHS.

(From the Laboratory of the Royal College of Physicians, Edinburgh.)

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Assistant Physician to the Skin-Department of the Royal Infirmary, Edinburgh.

Although within recent years numerous cases of multiple xanthomata have been recorded, and the disease is now a well-established entity, the following case presents several features of special interest. It is that of a girl, A. G—, aged 11 years, who was admitted on September 25th, 1909, to the Royal Hospital for Sick Children, Edinburgh, under the care of Mr. Harold J. Stiles, to whom I am indebted for permission to publish the case.

About three years ago a swelling was noticed on the middle finger of the right hand. About a fortnight later a similar swelling appeared on the middle finger of the left hand, and within the next few weeks several other swellings appeared on the hands. At the same time "lumps" were noticed on the arms, buttocks, and heels. These swellings and "lumps" all appeared somewhat suddenly, and rapidly reaching their present size, have remained stationary. They have never itched nor been painful, and have caused no inconvenience except on the fingers, where they interfere with the movements of the joints. The patient has always had fairly good health and attended school regularly. She has occasionally had attacks of giddiness.

Her father and mother are both alive and well. She has one sister and three brothers, all younger than herself, and says that

none of these show any swellings or skin-eruption similar to that seen on herself.

The patient has had no previous illnesses except measles, and has never been jaundiced. Her left foot was run over by a cart six years ago, when the wound was stitched by a doctor. Soon after the swellings appeared on her hands a "lump" was noticed on the scar of the former wound on her foot.

The patient is a somewhat delicate-looking child, but fairly well nourished. There is no sign of jaundice. There is no eruption on the face, neck, or body. Both hands (fig. 1) show rounded swellings projecting from the sides of the fingers. These are present on the ring and middle fingers at the level of the first intercarpal joint and over the knuckles of these fingers on each hand. There is also a similar swelling on the flexor aspect of the right thumb at the level of the centre of the first phalanx. All the swellings are covered with slightly reddened skin, which moves freely over them. They are fairly tense, somewhat lobulated, and at places give a feeling almost of fluctuation. They are not attached to the bones, and can be moved from side to side, but not vertically. They move with the tendons and are evidently attached to their sheaths. On the extensor aspect of the right elbow there is a raised yellow tubercle, which has a red margin. It is soft to feel, and is imbedded in the skin. There is a cluster of similar lesions over the prominence of the elbow of the left side (fig. 2). On the back of the left upper arm, just above the skin-eruption, there is a fairly tense, slightly lobulated swelling, about $\frac{3}{4}$ in. long by $\frac{1}{2}$ in. broad, which can be moved from side to side, but not vertically. This swelling feels like those on the fingers, and is covered with normal skin. On the buttocks (fig. 4) are several circular, flat lesions of a yellow colour, soft to feel, and embedded in the skin, and just above the anus is a small puckered depression in the skin, around which are several minute yellow spots. Over the right patella is a raised yellow spot, which is flat on the surface and about a quarter of an inch in diameter, and of the same character as the spots on the buttock. On the front of the right ankle and extending downwards on to the dorsum of the foot are similar flat, yellow spots, arranged in a circle, with a crenated edge and whitish-red centre. There are several large lobulated swellings, covered with normal skin, attached to the tendo Achillis and to the tendons of the

peroneus longus (fig. 3) of both sides, also a smaller swelling on the extensor tendon of the right great toe. There is a yellow mass in the centre of the scar on the outer border of the sole of the left foot (fig. 3). This mass has a crust in the centre, and looks as if it has been injured.

There is no enlargement of any of the lymphatic glands to be made out.

The liver is not enlarged. It extends from the upper border of the fourth rib in the mammary line to the costal margin. The lower edge cannot be felt.

The apex-beat of the heart is heaving in character, and situated between the fourth and fifth ribs just external to the nipple. There is a loud systolic murmur heard all over the heart, but best heard in the aortic area. This murmur is propagated up into the neck. The second sound is closed all over the heart and re-duplicated at the base of the heart. There is no sign of any enlargement of the aorta on percussion.

The urine contains no albumen and no sugar.

The patient was sent in October to the convalescent house at Gullane, but had to be re-admitted to hospital shortly afterwards on account of attacks of fainting and giddiness.

Now (December, 1909) the heart is in much the same condition as before, but the lower edge of the liver can be made out one finger's breadth below the costal margin. There is also a marked dilatation of the superficial veins over the lower part of the thorax and the abdomen. There are no signs of free fluid in the peritoneum.

On the first occasion on which patient was in hospital Mr. Stiles excised the skin-lesion from the right elbow and one of the tumour masses on the right middle finger. The latter was found to consist of a lobulated mass of yellow colour growing in the tendon sheath of the extensor tendon, and also in the prolongation of the sheath into the interpharyngeal joint, which had to be opened into in dissecting off the mass.

Part of this tissue was fixed in Müller's fluid, stained *en bloc* with 1 per cent. osmic acid solution and embedded in celloidin; part was fixed in formalin, and of these latter pieces some were cut with the freezing microtome, others were cut in celloidin in the ordinary way. The sections were stained with hæmatoxylin and eosin, methylene

blue and picro-fuchsin, hæmatoxylin and Sudan III, and Weigert's elastic tissue stain.

The structure of the lesion in the skin and tendon sheaths is essentially the same. Both are composed of a fibrous-tissue meshwork in which lie masses of cells (xanthoma cells) containing a fatty substance.

In the skin, which is otherwise unaltered, the papillæ are flattened out and the tumour-mass lies in the corium immediately below the epithelium (fig. 5). The mass has a liberal supply of blood-vessels, around which the cells are more closely packed, but contain less fatty substance than those further removed from the vessels. Most of the cells are definitely uninucleated with well-marked nucleoli, but there are groups of nuclei the outlines of whose cells cannot be definitely made out. These suggest more the close aggregation of several cells than the formation of giant cells. The fatty substance is in the cells, and consists of small droplets closely packed together. This fatty substance stains well with Sudan III. There is much less staining with osmic acid than one would expect from examining the Sudan sections. In the sections cut by freezing very numerous short crystals with bluntly pointed ends are seen (fig. 7). These are either single or arranged in bundles. They are most abundant where the fatty droplets are most numerous. In the skin the masses of cells have a fairly definite edge with finger-like prolongations into the surrounding tissue. In the tendon sheath the centre of the mass contains the large rounded cells full of fatty droplets, whilst the edge shows a gradual transition to normal fibrous-tissue parts showing fibrous tissue with rows of fatty droplets arranged in the connective-tissue cells. Elastic tissue is present in considerable quantity throughout the supporting framework of the mass.

Both clinically and microscopically, therefore, this case is a typical one of Xanthoma multiplex. Bodin (5), in *La Pratique Dermatologique*, subdivides cases of Xanthoma vulgaris into (1) Xanthoma planum; (2) Xanthoma tuberosum; and (3) Xanthoma "en tumeurs." In the case before us there is a mixture of all three subdivisions.

I do not propose to enter into any discussion as to the probable cause of xanthoma, as this case throws no light on the origin of the disease, nor to refer at all to the ordinary Xanthoma palpebrarum which is so often seen on the eyelids, and there only, nor to the

PLATE I.

Fig. 1

Fig. 2.

Fig 3.

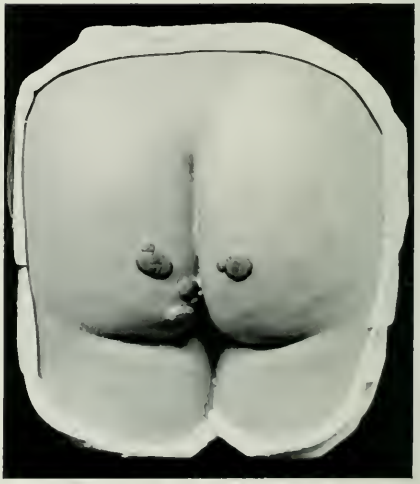

Fig. 4.

To Illustrate Dr. R. Cranston Low's Article on Xanthoma tuberosum.

PLATE II.

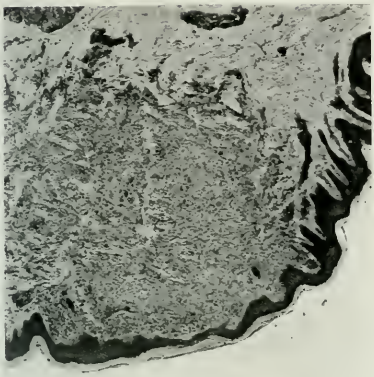

Fig. 5.

Fig. 6.

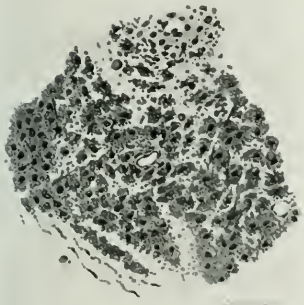

Fig. 7.

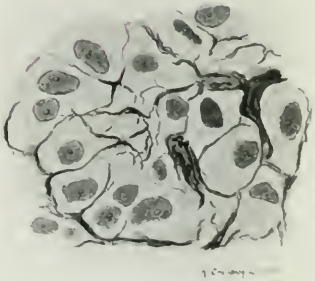

Fig. 8.

To Illustrate Dr. R Cranston Low's Article on Xanthoma tuberosum.

special form found in diabetes. The following remarks refer only to Xanthoma tuberosum multiplex.

Some authors have tried to separate the juvenile from the adult cases, but no such hard and fast line can be drawn between these two groups, as the disease is identical in children and adults, and cases are recorded at all ages from three months to sixty years of age.

The lesions in our case show a typical symmetrical distribution. The localisation on the elbows, hands, knees, buttocks, and feet is a very frequent one. The skin-lesions on the elbows at once suggest Xanthoma diabeticorum, but at no time has sugar been found in the urine. The absence of lesions on the eyelids is not unusual. In adults that situation is often the starting-point of the eruption, but several cases are recorded in children in which the eyelids have been early and extensively affected.

In many cases it is a family disease. Startin, Mackenzie (33), Hyde (29), Köbner, Hutchinson (28), and Thibièrge (46) have all reported cases where several members of one family were affected, either parent and child, or several members of both sexes in one family.

The special point of interest in our case is the presence of lesions on the tendon sheaths, in the heart, and possibly in the liver. There is a case reported in 1889 by Lehzen and Knauss (32) which is almost identical with our case. It was that of a girl, aged 11 years, who showed xanthoma lesions on the skin of elbows, buttocks, knees, and feet, and also had tumour masses attached to the extensor tendons of the fingers, the tendo Achillis, etc. This child had a mitral systolic murmur. Microscopically both the lesions in the skin and tendon sheaths were shown to be xanthoma. The child was suddenly seized with palpitation and breathlessness about three months after admission to hospital, and died within twenty-four hours. Post-mortem, yellow raised patches of xanthoma were found on the mitral valve, causing incompetence of the valve; also similar lesions were found in the aorta in one place in a ringed arrangement, causing considerable stenosis, in the left carotid artery, pulmonary arteries, and coronary arteries. The nine-year-old sister of the above case had similar but less extensive xanthoma lesions in the skin and tendon sheaths, but her heart was unaffected.

There is only one other case which I have been able to find where xanthoma lesions have been found post-mortem in the circulatory system. It is that reported by Pavy (36) and Fagge (15). Their case was an adult, who during life, in addition to showing skin and tendon-sheath lesions, had a mitral murmur, and post-mortem showed xanthoma of left auricle, aorta, pulmonary, innominate, and carotid arteries.

In addition to those examined post-mortem, a few others have been reported as having heart-murmurs in addition to generalised xanthoma of the skin. Poensgen's (38) case had stenosis of aorta, Pollosson's (40) case had a systolic murmur at the base of the heart, Vidal's (47) case mitral regurgitation, and Gwynne's two cases had mitral murmurs. Moxon reports a case of xanthoma where patches were found on the aorta. These were described as atheroma, to which condition the xanthoma lesions show a very close resemblance. Gwynne (20) gives photographs of his two cases, which showed lesions in skin and tendon sheaths very similar to our own. Both had heart lesions, and one of these children died very suddenly, and unfortunately no post-mortem was obtained.

Taking these facts into account with regard to heart-lesions in generalised xanthoma, I think that one is justified in coming to the conclusion that in our case the heart-murmur is in all probability due to xanthoma lesions in the aorta. Of course one cannot exclude endocarditis as the cause of the heart-murmur, but there is no history of rheumatism of any kind.

Lehzen and Knauss (32) recommend that the heart should always be examined in all cases of multiple xanthoma. In all such cases where a heart-lesion does exist a bad prognosis should be given.

Turning now to the lesions on the tendon sheaths, one finds that they are by no means so rare as the heart-lesions. After a careful search through all the available literature, I have been able to find records where the tendon sheaths (usually those of hands and tendo Achillis) were affected in seventeen cases. Those reported by Brachet and Monnard (6), Ehrmann (47), Fenlard (16), Gwynne (20), two cases, Hyde (29), Lehzen and Knauss (32), two cases, and Poensgen (38), were in children of twelve years and under. Those recorded by Addison and Gull (3), Chambard (11), Gottheil (19), Hardaway (24), Hillairet (26), Hutchinson (28), Pavy (36), and Fagge (15), occurred in persons over twelve years of age. There was no difference in the

lesions in the children from those in the adults. The diagnosis in such cases should cause no difficulty. The hands present at first glance (Fig. 1) an appearance like multiple chondromata, but the fact that the lesions are not hard and move with the tendon movements should be sufficient distinction. The associated skin-lesions should at once suggest a correct diagnosis. The only other lesions which the tumours on the hands suggest are dermoid cysts. Pollitzer (39) reports a case where there were several small tumours on the hands of a yellowish colour. These were diagnosed as xanthoma, but microscopic examination showed them to be multiple dermoid cysts. Pollitzer also refers to two somewhat similar cases recorded by Touton.

There was undoubtedly a slight increase in size of the liver in our case on the second occasion in which she was in hospital. The enlarged veins on the abdomen also suggested some obstruction. At no time has she been jaundiced; but as very numerous cases have been reported where the liver showed a cirrhotic condition, sometimes associated with xanthoma lesions obstructing the bile-ducts, one must admit the possibility of the commencement of some such lesion in our case.

The only other point to which I would refer in this case is to the microscopic appearances. Up till quite recently everyone has regarded the material found in the xanthoma cells as consisting of fat, but recently Pinkus and Pick (37) drew attention to the fact that, although this substance stains with Sudan, it is not an ordinary fat. They point out that it stains very poorly with osmic acid, and that the stain comes out when the specimen is put in xylol balsam. In our osmic acid specimens there is undoubtedly very much less of the fatty substance in the cells than in the Sudan specimens, but that we put down to incomplete fixation of the fatty substance before staining. The black staining is also rapidly fading, although the specimens are not more than a few weeks old. Pinkus and Pick consider that the substance in the xanthoma cells is a cholesterin fatty acid ester, and it differs from fat and fatty acids in being double-refracting under a polarising microscope. Pinkus and Pick also found that in absolutely fresh tissue there were no crystals, only droplets of the fatty substance, and crystals only appear after fixation in formalin. They consider the crystals and the droplets are two forms of the same substance. I

have examined sections from our case under a polarising microscope, and can confirm the observation that both the droplets and the crystals are double-refracting substances. Moxon (35) refers to these crystals as tyrosin, but microscopically, although they occur in bunches, they do not resemble the crystals of tyrosin.

The substance, cholesterin fatty acid ester, has been found in the blood in cases of jaundice and diabetes in appreciable quantities, and Pinkus and Pick suggest that it is through the circulation that the fatty substance reaches the xanthoma cells, where it is deposited, but they have no suggestion to offer as to why this substance should be in the circulating blood.

Description of Plates.

Fig. 1.—Hand showing tumour masses on tendon sheaths of ring and middle fingers. The small lesions in the skin are ordinary warts.

Fig. 2.—Back of elbow showing xanthoma in skin. Note also "lump" in triceps.

Fig. 3.—Sole of left foot showing xanthoma in scar on sole and tumour masses in tendo Achillis.

Fig. 4.—Buttocks showing skin-lesions.

Fig. 5.—Celloidin section. Low-power view of lesion in skin of elbow, showing mass of fibrous tissue stained with hæmatoxylin and eosin.

Fig. 6.—Frozen section of mass from tendon sheath of finger stained with hæmatoxylin and Sudan III, showing transition stage from fibrous tissue to typical "xanthoma"; droplets of fatty substance in connective-tissue cells.

Fig. 7.—Frozen section of centre of mass from tendon-sheath stained with hæmatoxylin and Sudan III, showing xanthoma cells with droplets of fatty substance; also crystals.

Fig. 8.—Celloidin section, skin of elbow; oil-immersion microscope; stained with methylene blue and picro-fuchsin, showing "xanthoma cells" lying in meshwork of fine fibres; vacuolated appearance of protoplasm where fatty substance has been dissolved out.

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EPITHELIOMA OF THE HYPOPHYSEAL DUCT.

By GEORGE MACKAY, M.D., Senior Ophthalmic Surgeon, and ALEXANDER BRUCE, M.D., Physician to the Royal Infirmary, Edinburgh.

(With Plates 11, 12 and 13.)

THE following case of tumour of the pituitary body seems sufficiently remarkable both from its clinical features and its morbid anatomy to warrant publication in the *Review*.

The patient, W. T., aged 16, clerk in a business office, was referred to Dr George Mackay on March 4th, 1908, by Dr William Stewart of Leith, and subsequently placed under the care of Dr Bruce. The history of the case, as obtained from the notes taken by the writers, is as follows :—

About six years previously some tubercular deposit about the lower end of the right femur was operated upon and removed by Dr Stewart. The patient made a good recovery, but had a limited power of flexion of knee and a slight limp. He had otherwise enjoyed fair health, though he was never very robust. In August 1905 he began to be troubled with headaches, without any obvious cause in the form of illness, accident or overwork. At first these were not constant, but from the beginning of 1906 they recurred almost daily, especially in the morning. The pain was generally at the back of the head, but sometimes came forward to the forehead. It was not accompanied by vomiting unless very severe. In the autumn of 1906 he entered a seed-merchant's office as clerk, but found the work too heavy. Three months later he was able to secure a situation in a wine merchant's office, where his duties were less confined and more varied, so that he was able to be more in the open air. For three months before coming to Dr Mackay he had noticed that his vision was defective.

March 4th, 1908.—External examination : Nothing abnormal save that the pupils were rather wide. They responded to light and on convergence. R.V. = 6/60, slightly improved with − 1D sph. Read J14 slowly. L.V. = 6/60, doubtfully improved with − 1D sph. Read J20 badly. Examination of the fields of vision revealed a complete temporal hemianopia of the *right* eye. Could neither count fingers nor even appreciate hand movements

on the temporal side of the middle line, while the nasal field was good for form and colour. With the *left* eye, fingers could not be counted, but hand movements were vaguely perceived in the temporal half field. In the *left* nasal field colours were correctly perceived, but neither perception of form nor colour seemed to be quite so acute as in the *right* nasal half field. The usual difficulty was experienced in mapping the actual line of demarcation between the affected and the unaffected parts near the middle line. Ophthalmoscopically—media transparent; both discs pale as if partial atrophy threatened.

On March 5th, 1908, at Dr Mackay's suggestion, Dr Logan Turner kindly examined the patient at the Royal Infirmary for possible evidence of trouble in the nasopharynx or accessory sinuses, but none was found.

On March 18th, 1908, he was admitted to the Eye Wards of the Royal Infirmary.

On March 20th he was referred to Dr Bruce, and lumbar puncture was performed, but no fluid was obtained.

On March 26th Calmette's ophthalmo-reaction was applied to his left eye, and on March 28th gave a positive result. There was no rise of temperature. The diagnosis of some tubercular disease, probably periostal in the sella turcica, seemed justifiable and was made.

The patient improved somewhat, had fewer headaches, and on dismissal, on the 17th of April, he was sent to the Convalescent Home for three weeks, taking cod-liver oil emulsion. He returned from there, looking much stronger and tanned with the sun, and he had put on three pounds in weight. His vision had also improved: R.V. with -1D sph. $= 6/36$. Read some of J12. L.V. with -1D sph. $= 6/18$ and J4. The improvement was maintained throughout the summer, and he returned to work.

In June 1908 he first noticed a peculiar discharge which sometimes came down the back of his nose. This appeared to be quite different from the usual nasal secretion, so that he always recognised it by the taste, but it had no disagreeable smell. It appeared every day, but usually not more than once a day, and when expectorated had a peculiar thick whitish appearance. This had continued to appear up till his admission to hospital. It is not increased in amount by any posture.

On October 16th, 1908: R.V. with − 1D sph. = 6/24 slowly. L.V. with − 0 5D sph. = 6/18 and some of 6/12. There was occasionally slight morning headache, but otherwise he was well and at work.

There is a history of his having fallen in the street in a fit, but details as to its nature and exact date are wanting.

On December 1st, 1908, his eyes again began to become weaker and his headaches to be more severe. He went back to see Dr Mackay on December 11th, 1908. On the 15th he saw Dr Bruce and was admitted to his ward on December 16th.

The following notes were taken by Dr Godwin Greenfield:—

On examination the patient was found to be a well-grown lad, tall but slightly built. The expression of the face was one of dulness and apathy. He seemed disinclined for conversation or any mental exercise, but was intelligent and evidently well-educated. There was slight head-retraction. The eyebrows were slightly raised, as if from headache, and the jaw, which was long and rather low-set, was kept slightly open. At intervals, when the pain was more than usually severe, he threw back his head and looked upwards as if in agony, while the mouth was rather more widely open than before. The eyes were sometimes opened wider than usual and sometimes half-closed during these attacks. They conveyed the impression of great pain of a wearing, exhausting nature. The eyes were steady, and except for a heavy, sleepy look, and more than usually long eyelashes, gave no evidence of his condition. The nose was well formed. There was no evidence of nasal catarrh, and no disagreeable odour was perceived in the breath.

The right knee-joint was almost completely ankylosed from old tuberculous disease occurring when he was five years old. The scars of sinuses were seen about the joint, which was slightly flexed and ill-shaped, with considerable thickening over its inner side. There was very considerable atrophy of the muscles of the right leg and thigh.

The chest was well formed, except for a flattening on the right lateral aspect, due to the use of a crutch since he was five years old.

The supra-clavicular fossæ, especially that on the left side, were slightly hollowed, and there was evidence at both apices of

tuberculous consolidation, which seemed to be progressing slightly at the right apex.

The heart and circulatory system appeared normal.

The urine showed no abnormal condition.

Examination of his nervous system showed complete bilateral hemianopsia, but no other contraction of the field of vision. Vision on the nasal side was poor, being $\frac{6}{18}$ in the left eye and $\frac{5}{60}$ in the right.

No paralysis or weakness of any of the external or internal muscles of the eye could be discovered. There was no paralysis of face or tongue. Except for the weakness of the right leg, due to the condition of the knee, the power of the limbs was intact. No change of sensibility of the skin could be made out. Taste and hearing were unimpaired. The sense of smell was very deficient in both nostrils, and was practically absent in the right.

The reflexes of the upper limbs were active, but not increased. No scapular jerk could be elicited. In the lower limbs the reflexes were much increased; adductor and crossed-adductor jerks were present on both sides. The left patellar reflex was much increased, but there was no great contraction of hamstrings following it. A slight ankle clonus, which, however, could not be sustained for more than a few contractions, was elicited on both sides, more on the left than on the right. Of the superficial reflexes, the abdominal and epigastric were very difficult to elicit. The cremasteric was active on both sides, and the plantar was active and flexor in character.

The special discharge from the nose could not be isolated for examination.

Examination by Dr Logan Turner in the ear and throat department revealed nothing abnormal in any of the anterior sinuses, and no evidence of disease was seen by either anterior or posterior rhinoscopy.

After admission to hospital he was kept quietly in bed for a week, during which time his general condition remained much as before. The spasms of great pain were not quite so frequent, although very severe when they came on. He slept well, had a good appetite, and felt himself rather better than he had been previously. He was treated with 5 grains of potassium iodide thrice daily, and full doses of cod-liver oil and malt.

On December 22nd, after a week's observation in hospital, lumbar puncture was performed under local anæsthesia at 1 p.m. The fluid came off at high pressure, a continuous stream pouring out to a distance of about 4 inches from the end of the needle, although this was inclined point downwards. After about 10 c.c. had come off in this way, he complained of very great pain in his head and desired the nurse to hold it, and there was marked head retraction. On account of the pain only 20 c.c. were drawn off. An ice-bag was applied to the back of his neck, and he was given 20 grains of potassium bromide. The headache remained very severe, and he lay groaning but quite conscious. The cerebro-spinal fluid was then examined and found to be highly albuminous and to reduce Fehling's solution slightly. It appeared quite clear, but yielded a very slight sediment on centrifuging for half an hour, which was of a reddish colour, composed mainly of red blood corpuscles, with an excess of leucocytes, of which the lymphocytes outnumbered the polymorphs by three to one.

About half an hour after the lumbar puncture the patient vomited, but after this remained fairly quiet all afternoon, although suffering great pain. He fell asleep about 8 p.m., but awoke again at 9.30, complaining of great pain at the back of his head. His head twitched back very frequently, and he seemed to become slightly flushed during these attacks. Kernig's sign was slightly present. At 10 p.m. he was given a powder containing 3 grains of exalgin, 3 grains of caffeine citrate, and 10 grains of phenacetin, which relieved the headache, and he slept for about an hour and a half. He awoke at 12 p.m., but had not at that time much pain, and lay awake until 3 a.m., when, as he was again complaining of much pain, 20 minims of nepenthe were given. After this he only dosed for the rest of the night, but had less pain.

On the following day he was given 20 grains pot. bromide thrice daily. When seen at 11 a.m., he complained of a good deal of pain in the head, and head retraction was fairly frequent.

Kernig's sign was rather more marked than on the previous night, the knee only being extended to an angle of 135°, and this caused him some pain.

He had a good deal of pain in the head all the afternoon, but took his food well. About 7.30 p.m. the pain became very

severe, so that he called out with it, and was given 30 minims of nepenthe, after which he slept soundly till 4.30 a.m., and fell asleep again shortly after.

On the 24th December he was very drowsy, and seemed to be sleeping nearly all the day. When roused up he said that his headache was much less severe. Head retraction was much less frequent, and he did not look so ill as on the previous day. He lay for the most part on the right side, with his head drawn slightly back, and the brow very slightly wrinkled. The eyes were shut, the eyebrows slightly raised, and the jaw hung slightly open. He slept soundly at night, waking only for short intervals, during which he complained of little headache.

On the 25th he was in much the same condition. When wakened and roused to speak he was quite rational and intelligent, but was usually very drowsy. He slept well again at night without any drugs.

There had been no difficulty with micturition up till this time, but the bowels were very constipated. From this time until the end he only passed urine when the bowels moved or from stimulation by hot cloths, and then in small amounts.

On December 26th he was very listless and drowsy, with the face slightly flushed and the tongue furred. He opened and shut his eyes listlessly, without seeming to take any note of his surroundings. There was slight difficulty in swallowing. He complained of no pain, and said that he was fairly comfortable. The pulse was rather faster, being 130 at 7 a.m.

When examined at 12.30 a.m. on the 27th the pulse was again slower—107 per minute. The eyes moved slowly from side to side, and the pupils reacted very sluggishly to light. The conjunctival reflex was diminished. The hands usually lay limply, but every now and again there were spasms running down the arm, causing a slight quivering. There was no evidence of paralysis of the face or limbs.

The reflexes were found as before, except the plantar reflex, which was now extensor in type. Head retraction was very slightly present, and Kernig's sign was more marked than previously. On account of the difficulty in swallowing, salines and whisky were given per rectum. At 2.30 a.m. the breathing became slightly Cheyne-Stokes in character; the pulse varied from 105 to 135, but was regular and of good volume.

At 3 a.m. he was given 3 oz. hot milk, which he took with difficulty, and went to sleep after it, and slept well until 4.15 a.m., when he awoke, complaining of pain. He dozed off to sleep again soon after, but at 5.30 was again awake, and complaining of his head. There were no twitchings of the head now, and no paralysis of the eye muscles could be observed.

At 7.45 a.m. he suddenly became very cyanosed, and died. There was no gurgling or gasping for breath, and death occurred in about three minutes.

Autopsy.—The base of the brain presented a very interesting condition. A large tumour, mainly cystic, was seen filling up the space between the tips of the two temporal lobes, the middle of the medulla posteriorly and the middle of the orbital surfaces of the frontal lobes. The cyst lay upon the optic chiasma and pressed upon it so that it could not be seen on the surface. It appeared to lie between the arachnoid and the surface of the brain. It had no apparent connection with the pituitary body, which merely lay upon the surface of its middle part. The surface of the middle part was nodular. A large cavity under the frontal lobe was opened during the removal of the brain and its contents escaped. The cyst wall had numerous minute nodules scattered over it, especially in the region of the chiasma. Their colour and firmness suggested that they were calcareous in nature (subsequent microscopic examination, see below, showed that they were really epithelial cell nests undergoing calcification). The membranes over the anterior surface of the pons and the medulla were thickened and a portion of the cyst extended backwards over the pons and medulla under the thickening of the arachnoid seen in Plate 11, Fig. 1. There was extensive absorption of the bone at the sella turcica, so that the sphenoidal cells were merely roofed in by mucous membrane, which Dr J. S. Fraser, who kindly examined it, reported to be practically normal. The nasal mucous membrane was in a state of catarrh. No affection of the spinal cord was discovered. Both lungs showed evidence of early phthisis at the apices.

Further examination of the brain.—On mesial section a tumour about 8 cm. long and 4·2 cm. in greatest breadth was seen occupying the whole of the third ventricle, displacing its lateral walls outwards, destroying both septa lucida and extending forwards so as to displace the orbital surface of the posterior

part of the frontal lobe upwards. As shown in Fig. 2, its backward growth had displaced the crura cerebri and pons in such a manner that their posterior surface was almost at a right angle with the corpus callosum.

The upper part of that portion of the tumour which lay in the third ventricle was occupied by several large gelatinous masses of irregular outline. Two of these, which measured from about 18 to 20 mm. in their longest diameter, with intermediate smaller cysts, are well seen. They resembled the colloid substance in cystic tumours of the thyroid, but their nature, as will be seen later, was totally different.

The anterior portion of the lower half was occupied by a single large cyst, the contents of which, as already mentioned, escaped at the examination. Its lining membrane was studded irregularly by minute, opaque, whitish granules. (These can be seen with the aid of an ophthalmoscopic lens in Fig. 2.) The posterior part of the lower half of the tumour consisted of small cystic cavities with broad intervening trabeculæ which presented partly a gelatinous appearance and partly fine opaque whitish granules of apparently calcareous matter. These granules appear as the whitish portion between the large cyst in the posterior and the multilocular cysts in the anterior portion of the tumour. The part of the tumour which lay in front of the pons and medulla was a flattened cyst, which collapsed on being opened.

Microscopical examination.—The pituitary body itself (Plate 12, Fig. 4) seems quite normal. The following description of the tumour is taken from that part described as the posterior portion of the lower half (Fig. 2). Under the low power (Plate 12, Fig. 3) the growth resembles a cystic adenoma, but a careful examination shows that the cyst-like spaces, lined by an almost cylindric epithelium, are really formed by the connective tissue stroma between branching epithelial ingrowths — the several branches of which anastomose with one another. This connective tissue stroma is in all stages of mucoid degeneration (Fig. 3*a*, *a'*), so that "spaces" are formed, some with almost normal fibrous tissue, others with branching myxomatous cells, swollen and separated fibrils, and a mucoid ground substance, and still others where the mucoid transformation has been complete. It is these last which give at first sight the impression

of a cystic tumour, but even these contain well-formed capillaries (Fig. 3*b*), which stand out clearly in the mucoid substance. A few of the spaces contain numbers of large, vacuolated, rounded cells. These are probably of a phagocytic nature, and are to be found to a smaller or greater extent in all the spaces, often grouped round capillaries (Fig. 5*a*). In a few of the larger spaces the mucoid material has either fallen out or been absorbed, and only swollen strands of connective tissue fibrils are left, together with a few lymphocytes and large granular cells (Fig. 3*a'*).

The branching epithelial ingrowths (*c c*,) show all stages of complexity, from the simple two-layered process covered only by the original germinal epithelium which adjoins the connective tissue to the ingrowths, consisting of several layers of epithelial cells, the outermost of which represent the germinal epithelium, and the inner ones, becoming flattened and cornified, form typical cell-nests (Fig. 3*d* and Fig. 5). In many of the spaces are found islands of epithelium showing all stages in the formation of cell-nests, many going on even to calcification (Fig. 6).

The calcareous granules in the wall of the large cyst which underlay the frontal lobe and formed the anterior portion of the lower half of the tumour proved to be epithelial cell-nests in process of calcification.

It will thus be seen that the diagnosis of the site of the lesion, but not that of its nature, was confirmed. The bitemporal hemianopia seemed to put beyond doubt that there was a pressure on the optic chiasma. On the other hand, the evidence obtained by Calmette's reaction was altogether deceptive. Its results merely indicated that the patient was tuberculous ; they did not tell where the tuberculosis was situated, and in this case it was not in the brain, but was in the lung. The two conditions—one of epithelioma, the other of tuberculosis—co-existed ; the tuberculosis in the lung spoke, but the tumour of the hypophysis was silent.

As already stated, the pituitary body itself was found to be quite normal both as regards its glandular and its nervous portions, as well as in the lining membrane and form and contents of the epithelial cleft. No connection beyond that of a fibrous-tissue nature could be made out between the pituitary body and the tumour. Although no further connection could

be traced, it appears to us probable that the tumour must have arisen from some aberrant portion of the wall of that part of the ectoderm of the original embryonic buccal cavity which goes to form Rathke's pouch. The epithelial cells lining this pouch, being derived from the ectoderm, would be imbued with the potentiality of developing in the same manner as the latter—that is to say, as squamous epithelium—and this although all trace of any of the extra-pituitary portion of Rathke's pouch was ultimately lost.

Such forms of tumour seem to be comparatively rare, and the writers would refer to the paper by Erdheim (*Sitzungsberichte der Kaiserl. Akad. der Wissenschaft, Math.-naturw. Klasse*, Bd. 113, H. 10, Dec. 1904, Abt. 3).

The absence of acromegaly in such a case as this and those described by Erdheim is not to be wondered at, seeing that the tumour did not involve or arise from the pituitary itself.

The microscopic sections in this case were made by Dr J. W. Dawson, and their photographic reproduction by Mr William Watson, of the Royal College of Physicians' Laboratory.

Description of Plates.

Plate 11.

Fig. 1.—Base of brain.

a. Large cyst in anterior part of the tumour, opened in its removal.

b. The nodular under surface of the part of the tumour lying in front of the pituitary body.

c. The pituitary body. Behind it, over the pons and upper part of the medulla, is a thickening of arachnoid which corresponded to the hindmost portion of the cyst.

Fig. 2.—Mesial surface of left hemisphere.

The tumour, as described in the text, has displaced the cerebral peduncle and pons into an almost vertical position.

Plate 12.

Fig. 3.—Section of tumour.

a, *a'*. Stroma in various stages of myxomatous degeneration.

b. Capillaries in stroma.

c, *c*. Epithelial ingrowths.

d. Cell-nest.

FIG. 1.

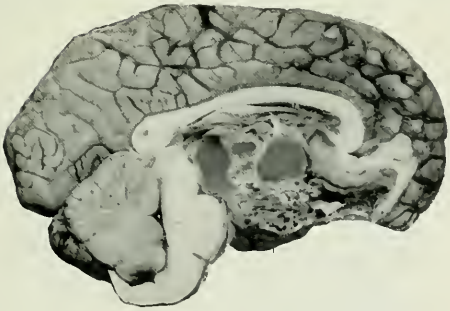

FIG. 2.

To illustrate Paper by Drs Mackay and Bruce.

FIG. 3.

FIG. 4.

To illustrate Paper by Drs Mackay and Bruce.

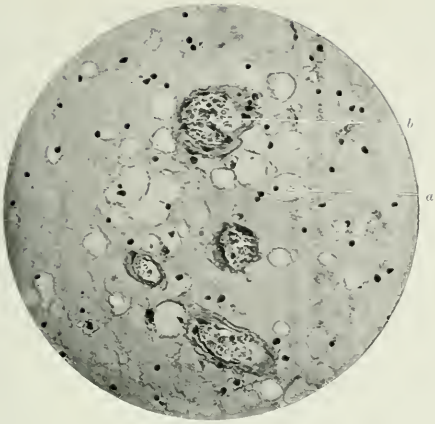

FIG. 5.

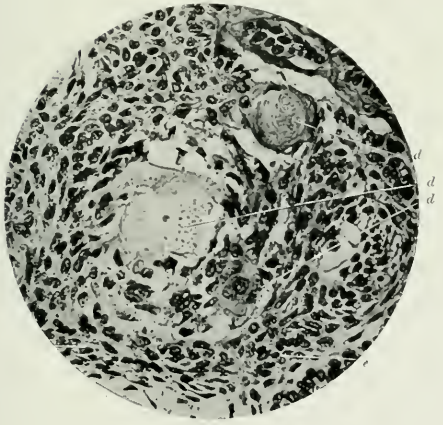

FIG. 6.

To illustrate Paper by Drs Mackay and Bruce.

Fig. 4.—Section of pituitary body (L.P.), showing—to the left side, the epithelial ; to the right side, the nervous portion ; in the middle, the epithelial cleft. All these appear normal.

PLATE 13.

Fig. 5.—Section of mucoid stroma, showing phagocytic cells (absorbing mucoid substance ?) near capillaries.

Fig. 6.—*d*, *d*, *d*. Cell-nests.

e. Several layers of epithelial cells.

CHRONIC INFLAMMATORY ŒDEMA OF THE SUB-MUCOUS TISSUES OF THE NOSE

BY

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1908.

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CHRONIC INFLAMMATORY ŒDEMA OF THE SUB-MUCOUS TISSUES OF THE NOSE

By J. S. Fraser, M.B., Ch.B., F.R.C.S.Ed.,
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Formation and Functions of the Nose

The nasal septum is seldom or never absolutely straight, in fact a straight septum is an abnormally normal one; the outer wall of the nose is thrown into convolutions by the turbinals so as to expose as much secreting surface as possible in the small space available. The inspired air entering the nasal chambers thus strikes against the septum or turbinals before passing back to the naso-pharynx, and is warmed, moistened, and filtered to some extent from dust particles and bacteria before it reaches the cavities of the pharynx and larynx. The nasal mucous membrane is likely, therefore, to be a very active structure; it is well supplied with blood-vessels, and in certain parts, notably in the inferior turbinal region, these vessels take part in the formation of erectile tissue; the size of the nasal cavities can thus be regulated according to the state of the air entering the nose; the inferior turbinals swell up in cold weather or in dusty atmospheres; excitement also influences the state of engorgement of the turbinals, and the well-known phrase, "open-mouthed with astonishment," would be more accurately replaced by "shut-nosed with astonishment." The size of the inferior turbinals may also be seen to vary during the short period covered

by an examination of the anterior nares, and we are probably all familiar with the fact that if we lie on one side in bed the nostril on that side tends to become blocked.

Method of carrying out the Present Investigation

The number of specimens examined was 70; of these 22 were inferior turbinals, of which 6 were normal, 6 in a condition of slight œdema, 5 markedly œdematous, and 5 in the condition known as "cauliflower hypertrophy." This state, however, is merely one of very extreme œdematous infiltration. Eighteen specimens of the middle terbinal were examined, in addition to 30 which formed the subject (1) of a previous paper. Of the middle terbinals 3 were normal, 10 showed slight œdema, and the rest marked œdematous hypertrophy, or, in other words, nasal polypus formation. In all but a few cases the tissues were removed during life, washed in water to remove blood and mucus from the surface, and placed at once in 5 per cent. nitric acid in spirit. After a week they were transferred to 10 per cent. nitric acid in spirit and changed three or four times according to the size of the specimen. The time required for decalcification varied in specimens which contained apparently an equal amount of bone. The tissues were carried through in the usual way and embedded in paraffin and cut often in serial sections. It was found by no means easy to get good sections of tissues of such varying degrees of density as the turbinals, containing bone, periosteum, loose connective tissue glands, and delicate mucous membrane. The staining method used was the ordinary one—hæmatoxylin and eosin, but Unna's orcein method was used in addition in almost all cases for elastic fibres.

Sites of Chronic Inflammatory Œdema in the Nose

(A) *The Septum Nasi.*—(1) Anteriorly in the region of the tubercle; (2) posteriorly. Swelling is often seen by posterior rhinoscopy, apparently in the region of the posterior border of the vomer high up towards the roof of the choanæ, giving rise to an appearance that may be compared to the "ace of hearts." This is probably merely an extension backwards of the œdema in the region of the tubercle.

(B) *Middle Turbinal.*—The anterior end, lower border, or outer surface are most frequently affected; these parts are the most dependent.

(c) *Inferior Turbinal.*—The anterior end is affected rather more often than the posterior end, but frequently the anterior end, inner surface, and lower border, as well as the posterior end, are all affected.

Normal Structure of Inferior and Middle Turbinal Bodies

Inferior Turbinal.—(Fig. 1). This consists of a bony framework with an irregular surface; within this we find so-called marrow spaces lined by delicate connective tissue and containing a central thin-walled blood-vessel. The periosteum consists of two or three layers of elongated cells, and just outside this there is a dense layer of elastic tissue from which strands of elastic fibres run out radially in all directions towards the mucous membrane (Fig. 7). Beyond

Fig. A

the periosteum we have a layer of fairly dense connective tissue containing the larger blood-vessels and some unstriped muscle-fibres, and outside this again the erectile tissue-layer made up of thin-walled blood-vessels of irregular size and shape; the erectile tissue is better marked at both ends and along the inferior border of the turbinal. Just under the superficial epithelium and its basement membrane we have the racemose mucous glands (Fig. 2), the ducts of which open through the basement membrane usually into smaller hollows on the surface. Immediately under the basement membrane there is a thin layer of elastic fibres running parallel to the surface but connected with the fibres that run out radially from the periosteum; the whole turbinal has thus a framework of elastic fibres which causes it to return to its normal size when vascular engorgement is over. The covering layer consists of ciliated columnar epithelium, beneath which are several layers of cubical or irregular cells situated upon the basement membrane.

Blood supply: The larger vessels run near the periosteum more or less parellel to the long axis of the turbinal; from these smaller vessels run radically outwards to the blood sinuses and to the mucous

glands, and, finally, very small vessels ramify just under the basement membrane.

Normally in living people there is always a certain amount of small-cell infiltration under the basement membrane and around the glands and superficial blood-vessels; it is only in the unborn that this is not found (Kubo).

Middle Turbinal.—(Fig. 6.) This is much the same as that of the inferior turbinals, but with the following differences: the bone is thinner and altogether more delicate; not infrequently there is a definite cell in the middle turbinal lined with ciliated epithelium, in fact, in these cases the middle turbinal contains one of the ethmoidal cells. The erectile tissue is not so marked in the middle turbinal as in the inferior, and the whole structure is looser and more delicate. From an anatomical point of view we must remember that the middle turbinal is situated higher up in the nose than the inferior.

Microscopical Appearances in Cases of Œdema of the Middle and Inferior Turbinals

(*a*) *The Inferior Turbinal.*—After writing a paper on "The Ætiology of Nasal Polypus," it occurred to me that further light might be thrown on this question by the examination of the inferior turbinal bodies, and I was not surprised to find that the changes leading to so-called hypertrophy of the *inferior* turbinals were exactly similar to those seen in the middle turbinals in cases of nasal polypus formation.

It is necessary in the first place to distinguish between engorgement of the inferior turbinals due to distension of the blood spaces (cavernous tissue) and œdema of the submucous tissue (so-called hypertrophy of the inferior turbinals). This can readily be done by the application of cocaine to the turbinal; vascular engorgement goes down within a few seconds, whilst œdema is little, if at all, affected. In some cases both conditions are present, and, on the application of cocaine, the turbinal shrinks to some extent, but still remains considerably enlarged. It is extremely probable, however, that chronic engorgement is the first stage of inflammation, leading to œdematous infiltration when changes have occurred in the walls of the blood-vessels. In early cases of œdema of the inferior turbinal, the process commences just under the basement membrane, which is frequently thickened (Fig. 3). Kubo considers this thickening to mean inflammation; the leucocyte infiltration is increased,

especially round the vessels and glands, and, in some cases, amounts almost to the formation of lymph nodules. The glands are slightly pushed inwards by the œdema and the ducts dilated; the blood spaces, periosteum, bone, and marrow are not affected.

As the œdema increases (Fig. 4) the glands and blood spaces are pushed further back, and the surface of the turbinal tends to become crenated or scalloped, as seen in sections—papillary, as seen on surface view. This is due to the gland ducts tacking down the mucous membrane at various spots, and can be well seen in the posterior ends of the inferior turbinals by posterior rhinoscopy in cases of so-called "mulberry hypertrophy."

In the œdematous submucous tissue there are a large number of cells showing myxomatous degeneration, and it is due to this fact that nasal polypi were so long regarded as myxomata.

Even in the most marked conditions of œdema of the inferior turbinal—the so-called "cauliflower hypertrophy"—the surface epithelium is but little changed (Fig. 5) and the cilia are well preserved; here and there leucocytes may be seen making their way through the superficial epithelium to the surface; this fact accounts for the muco-purulent discharge present in so many of these cases.

If we examine one of the papillæ on the surface of such an inferior turbinal under a high power, we are at once struck by the close resemblance to a nasal polypus.

The changes in the inferior turbinal are more marked on the inner surface, *i.e.* the suface next the septum over which the air current passes (Fig. 3).

In advanced cases there may be some thickening of the periosteum, but the bone and marrow spaces are always normal so far as my observations go. Finally, I have noticed that in these cases the blood spaces are usually small; I have remarked before that they are pressed towards the bony centre of the turbinal by the œdema which occurs between them and the mucous membrane, and this fact probably accounts for their diminution in size.

(*b*) *Middle Turbinal.*—The first change noted is œdema of the connective-tissue layer just under the basement membrane (Fig. 8); the connective-tissue spaces are enlarged and contain serum, and the glands and blood spaces are gradually displaced inwards towards the bony centre of the turbinal as the œdema increases. The epithelial covering of the turbinal, along with the basement membrane, is of course pushed away from the bony framework,

and later on comes to hang down in the nasal cavity; this is the condition we recognise clinically as "polypoid middle turbinal." The ducts of the glands in many cases become obstructed—on account of the œdematous infiltration—and in this way large cystic spaces are developed.

It is very difficult to speak with certainty as to the condition of the mucous membrane; during the course of hardening, decalcification, embedding, or staining, it often happens that the surface epithelium gets separated and lost; but it is also remarkable that in cases of marked nasal polypus formation the superficial epithelium is apparently quite normal.

Causes of Œdema

Without doubt the two most common pathological conditions affecting respectively the middle and inferior turbinals are nasal polypus and inferior turbinal hypertrophy (so called). Differences in the macro- and microscopic appearances are due to, *firstly*, difference in the structure of the two turbinals, and, *secondly*, difference in anatomical position, or, in other words, the middle turbinal is of looser and more delicate structure and situated further from the floor of the nose.

In both cases the cause of the œdema is to be found in repeated or severe attacks of acute catarrh (coryza): in this condition the turbinals are hyperæmic and slightly œdematous, but as a rule resolution occurs and the parts return to normal: on the other hand in a few cases the inflammation becomes chronic, and the deeper layers of the mucous membrane are involved. Clinically, these chronic cases are characterised by constant nasal discharge (mucous or muco-purulent) and nasal obstruction: the patients complain that they are never free from a "cold" in the head, and they usually state that they have suffered from nasal discharge and obstruction for several years. The tissues of the nose are thus constantly soaking in their own secretions, and a return to normal is prevented. It has been pointed out to me that an analogy may be found in the case of papilloma of the bladder: this condition is so serious because it is impossible to keep the parts dry, whereas warts on the hand tend to shrivel up and disappear when moisture is excluded.

On going over the cases of inferior turbinal enlargement which presented themselves at Dr Logan Turner's clinic during the year

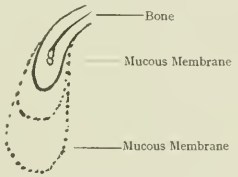
1907, I found that the actual number was 242, but the great majority of these were cases of vascular engorgement. In most of the cases there was only slight enlargement, but it was noted that in 30 there was a co-existing polypoid condition of the middle turbinals, and probably this number would have been greater had cocaine been applied in all instances to the inferior turbinals so as to obtain a better view of the middle turbinal region. Of the 79 cases classified under the head of nasal polypus, there were 20 with co-existing enlargement of the inferior turbinal.

Formation of Polypi

Œdema occurs beneath the mucous membrane along the lower border or outer surface of the turbinal: the mucous membrane thus sags down in the nose, and in advanced cases reaches the floor of the nasal cavities, or even protrudes from the anterior or posterior nares.

Before this stage is reached, however, the developing nasal

Fig. B.



polypus is acted upon by the respiratory air currents, and its base of attachment is specially dragged on in the act of blowing the nose: gravity also tends to increase the formation of the œdematous swelling once it has begun. From what has been said it is not difficult to understand that in some cases the base becomes constricted so that a pear-shaped swelling results, but in my opinion the genuine pear-shaped polypus is much less frequent than is generally believed. I hold that the loop of the snare is frequently responsible for the pear shape, and that in most cases the polypus is broad based along the lower border or outer surface of the turbinal. If we think of the frequency of œdema in inflammation of such loose structures as the prepuce, scrotum, and lower eyelid, we need not wonder that it occurs in the chronic inflammation of the middle turbinal region of the nose. In the inferior turbinal the case is somewhat different; here the tissue is denser, and the lower border

of the turbinal is already almost on the floor of the nasal chamber; consequently we do not meet with such marked œdematous swelling. At the *posterior* end of the inferior turbinal we may, however, in advanced cases, see a large papillary mass of œdematous tissue hanging down in the posterior surface of the soft palate.

Inflammation in any situation is always associated with exudation, and the looser the tissue the greater the amount of exudation. I believe that in early cases of rhinitis we have an excessive amount of vascular turgescence associated with a slight degree of œdematous infiltration; this goes down under cocaine application. If the inflammation becomes chronic the œdema increases most probably on account of changes in the walls of the blood-vessels and blood spaces. Lymphatic and venous obstruction no doubt play a part, and I have observed swelling of the endothelical cells lining the lymphatic vessels in one or two of my specimens. Watson Williams (3) recently showed a specimen of nasal polypus in which a lymphatic vessel appeared to be blocked by inflammatory products. Hopman (4) thinks that the cause of the œdema is some impairment of the circulation in the efferent venous vessels, and the engorged state of the small veins in many of the specimens examined by me supports this theory (Fig. 12). Grünwald (5) explains the œdema by changes in the vessels of the nature of peri- and endo-arteritis, but one is bound to note that even in apparently normal turbinals the vessel walls seem to be abnormally thick. Goodale (6) says, "It is possible that the inflammatory infiltration which penetrates the substance of the turbinals may lead in some places to a stasis from compression of the veins"; the same writer entirely disagrees with the bone disease theory of nasal polypus formation.

In advanced cases the elastic fibres are hard to find—at least in the œdematous parts; near the periosteum they are present and normal in appearance. The striking similarity of the changes in the middle and inferior turbinals will be apparent from the illustrations, and nobody has yet said that inferior turbinal œdema or hypertrophy is due to bone disease; we may well ask why œdema of the middle turbinal should be due to this cause. It is true that the bone is often affected in advanced cases of nasal polypus formation, associated with ethmoidal suppuration, but in this case the bone disease is *the effect*, not the cause (Fig. 11). Yonge's (7) theory a to the causation of nasal polypi was not well received at the last meeting of the British Medical Association at Exeter, and as I have dealt with it in a previous paper I need not do so again.

FIG. 1.—NORMAL INFERIOR TURBINAL: the bony core of the turbinal is over-decalcified, but the figure shows the large blood-vessels in the periosteal layer, the blood spaces best marked along the lower border and inner surface, the numerous glands just under the basement membrane, and the superficial epithelium (absent in parts). Note the small cell infiltration of superficial layers most marked on inner surface. × 6 diam.

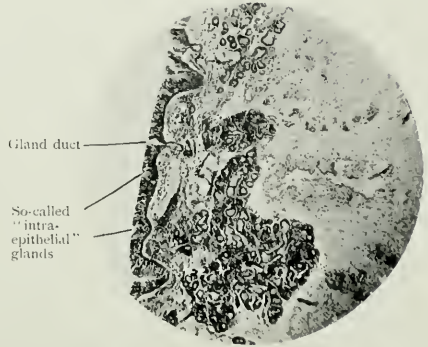


FIG. 2.—NORMAL MUCOUS MEMBRANE of inferior turbinal. Note superficial epithelium containing what appear to be "intra-epithelial" glands; this appearance may be due to oblique section of a gland duct; small cell infiltration (1) of superficial layers, (2) between gland acini. × 50 diam.

FIG. 3.—EARLY INFLAMMATORY ŒDEMA of the inferior turbinal; superficial epithelium is absent; note that the glands are slightly pushed inwards towards the bony core of the turbinal. The œdema of the septal surface of the turbinal is shown when contrasted with outer surface where the tissues are almost normal. × 6 diam.

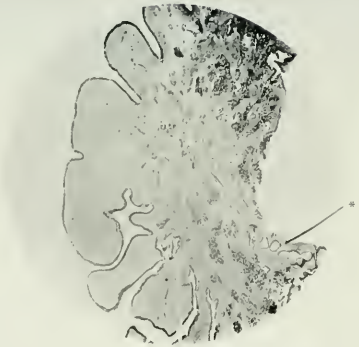


FIG. 4.—Marked chronic inflammatory œdema of the inferior turbinal; "cauliflower hypertrophy"; the glands markedly displaced inwards; dilated ducts are seen opening into the hollows between the papillæ in the lower part of the section; the blood spaces are few and small; the bony core of the turbinal is, however, quite healthy; the walls of the blood-vessels appear to be considerably thickened. × 6 diam.

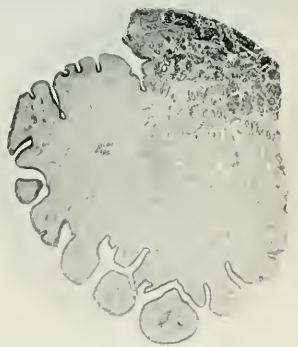


FIG. 5.—Marked chronic inflammatory œdema of posterior end of inferior turbinal. Notice œdema of superficial layers and displacement of glands and blood spaces. × 6 diam.

In all cases the microscopic section has been made in the coronal plane.

PLATE VII.—TO ILLUSTRATE DR J. S. FRASER'S PAPER.

FIG. 6.—Normal middle turbinal. Note the more delicate structure of the middle turbinal; the glands are numerous and quite near to the surface; the blood spaces are small and fewer than in the inferior turbinal.
× 6 diam.

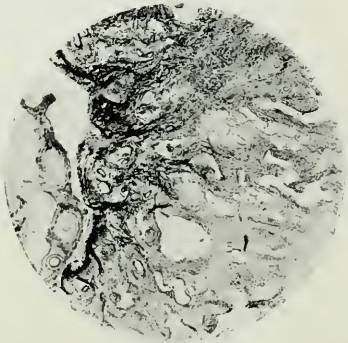


FIG. 7.—Elastic tissue of the middle turbinal. Around the periosteum the fibres form a dense network from which more delicate strands radiate out into the substance of the turbinal surrounding the blood spaces and glands; just under the basement membrane there is a delicate layer connected to the radiating fibres. × 50 diam.

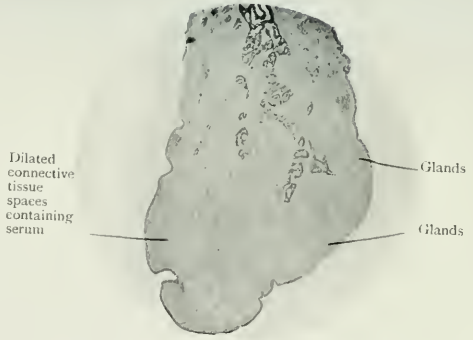


FIG. 8.—Early CHRONIC INFLAMMATORY ŒDEMA of the middle turbinal, or POLYPOID middle turbinal. Note large connective-tissue spaces containing serum along the lower border of the turbinal; the glands in this region are displaced inwards; the bone is healthy. × 6 diam.

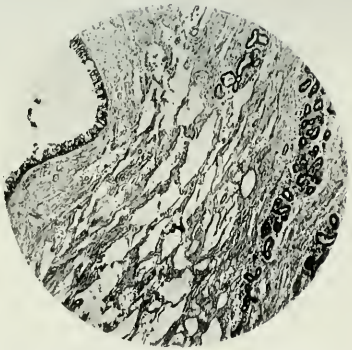


FIG. 9.—Higher power view of same specimen as shown in Fig. 8. Note glands pushed inwards by œdema of superficial layers. × 50 diam.

In all cases the microscopic section has been made in the coronal plane.

PLATE VIII.—TO ILLUSTRATE DR J. S. FRASER'S PAPER.

CHANGES IN THE MIDDLE TURBINAL—*Continued.*

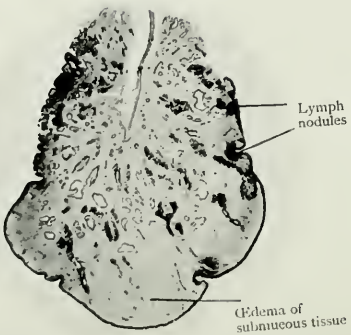


FIG. 10.—Slight chronic inflammatory œdema of the middle turbinal; slight dilatation of gland ducts; inward displacement of glands and blood spaces. Note marked small cell infiltration of superficial layers.

FIG. 11.—Œdema of middle turbinal in case of ETHMOIDAL SUPPURATION. The normal tissues are crowded towards the bone; the thickened lining membrane of the cell in the middle turbinal is well seen, and the small cell infiltration of its mucous membrane. × 6 diam.

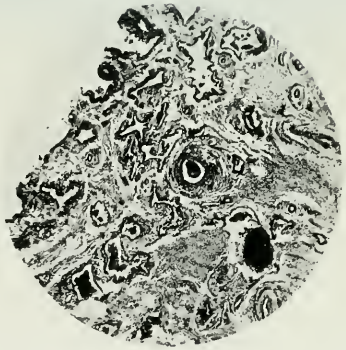


FIG. 12.—This section shows the appearance of the tissues at the base of the polypoid swelling. Note the dilatation of the lymphatic vessels and spaces. In the centre of the field a blood-vessel is seen containing organised tissue—probably a thrombus.
× 50 diam.

FIG. 13.—Section of TYPICAL "NASAL POLYPUS," showing large connective-tissue spaces filled with serum, dilated gland ducts and small blood-vessels; the superficial epithelium is almost normal.
× 6 diam.

In all cases the microscopic section has been made in the coronal plane.

PLATE IX.—TO ILLUSTRATE DR J. S FRASER'S PAPER.

In regard to Grünwald's theory that nasal polypi are associated with sinus suppuration, I can only state that this appears to be true in only 50 per cent. of cases. I think we are not far wrong if we regard the character of the discharge present as diagnostic—that is to say, if nasal polypi are found associated with a clear watery discharge no sinus suppuration is present; if the discharge is muco-purulent, sinus suppuration may or may not be present; if the discharge is distinctly purulent then sinus suppuration is present.

Morell Mackenzie used to urge against the inflammatory origin of nasal polypi that while nasal catarrh is very frequent in children, polypi are extremely rare. There are two good answers to this objection: (1) the middle turbinal region is not easy to examine in children, and minor degrees of œdema may easily pass unnoticed; and (2) it is extremely probable that the vascular changes leading up to marked polypus formation take years to occur—in other words, children have ceased to be children when polypi are developed.

In conclusion, I wish to express my thanks to Dr Logan Turner, who has kindly allowed me to make use of his clinical material, to the Royal College of Physicians (Edinburgh) for permission to work in their Laboratory, and to Dr James Ritchie for much advice and assistance in carrying out the work.

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(1) *Scot. Med. and Surg. Journ.*, April 1907.
(2) *Archiv. f. Laryngologie*, Bd. xix. S. 202.
(3) *Proceedings of the Royal Society of Medicine*, 1908.
(4) *Wiener med. Presse*, 1883.
(5) "Nasal Suppuration," 1900.
(6) "Diseases of Eye, Ear, Nose and Throat," by Posey and Wright, p. 706.
(7) "Polypus of the Nose," 1906.

THE HISTOLOGY OF NASAL ACCESSORY SINUS SUPPURATION. A COMPARISON OF THE SIMPLE INFLAMMATORY PROCESSES IN THE UPPER RESPIRATORY TRACT—INCLUDING THE MIDDLE-EAR CLEFT

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The object of this paper is to show that the changes which occur in the mucous membrane of the accessory sinuses in simple acute and chronic inflammatory conditions are analogous to those which take place in the nose in catarrhal and suppurative rhinitis, and in the middle ear cleft in catarrhal and suppurative otitis media; secondly, to bring forward evidence in favour of the view that these processes occur first of all in the nose and affect the pharynx, accessory sinuses, and middle ear by direct extension, and further that there is no essential difference between what is called "catarrh" and what is called "suppuration," as one condition passes insensibly into the other.

Under normal conditions the accessory sinuses contain air, while the lining membrane is coated with a delicate layer of mucous

secretion: it is important to remember than the cavities are lined by mucous membrane continuous through the ostia with that of the nasal cavities. We are all aware of the fact that an attack of coryza is frequently followed by more or less catarrhal pharyngitis, laryngitis, and even tracheitis, and it seems probable that the mucosa of the sinuses takes part in any changes which the mucous membrane of the nose may undergo. Harke, E. Fränkel, and Oppikofer on the one hand think that sinus suppuration is the direct consequence of the causative disease, whereas Zuckerkandl, Hajek, Lack, and Goetjes are of opinion that most cases are due to the spread of a catarrhal process from the nose. It is hardly necessary to say that the accessory sinuses are not always affected by an attack of acute rhinitis, just as the middle ear cleft is not involved in every case of measles or scarlet fever. On the other hand, it frequently happens that although the catarrhal process in the nose may have subsided, the inflammatory condition has continued in the maxillary antrum or in the tympanic cavity. It has been frequently stated that cases of maxillary antrum suppuration, which have been treated and apparently cured by alveolar puncture, often relapse during an attack of coryza, and this fact supports the view that the mucous membrane of the sinuses takes part in the inflammatory processes which have invaded the nasal cavities. In regard to the physical conditions which obtain in the accessory sinuses, it is necessary to call attention to the fact that the ostia are very small in proportion to the size of the cavities they drain, and further that these ostia are, in the case of all except the frontal sinus, very badly situated for drainage by gravitation. Under normal conditions drainage is probably carried on by ciliary action alone, but in diseased conditions the epithelium is not infrequently damaged, and under such circumstances gravitation, syphonage, and suction may come into play (Yankauer).

Histology of the Normal Mucosa of the Accessory Sinuses.—In dealing with specimens obtained from the post-mortem room, it is an exceedingly easy matter after formol fixation to strip the mucous membrane from the underlying bone with a small blunt dissector, such as that used in the submucous resection of the septum; and even during operation on a diseased sinus it is often possible to detach large areas of thickened mucosa in the same way. Oppikofer lays stress on the examination of large pieces, as varying pathological conditions may be found at different parts of the mucous membrane obtained from the same sinus: this statement is confirmed by

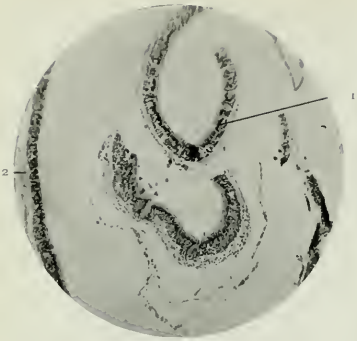


FIG. 1.—Normal mucous membrane from maxillary antrum. × about 50 diam. 1. Ciliated mucous membrane. 2. Submucous connective tissue.

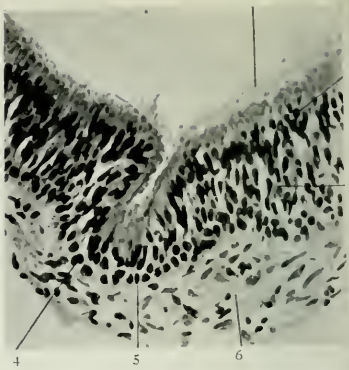


FIG. 2.—Normal mucous membrane from maxillary antrum. × about 400 diam. 1. Normal mucous secretion. 2. Superficial layer of columnar ciliated cells. 3. Intermediate layer of spindle-formed cells. 4. Deep layer of cubical cells. 5. Basement membrane. 6. Connective tissues.

FIG. 3.—Normal ostium of sphenoidal sinus. × about 17 diam. 1. Epithelial lining of sinus. 2. Mucous glands in connective tissue.

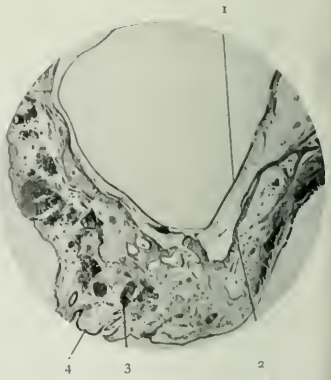


FIG. 4.—Normal middle turbinal, showing air-ce lined by healthy mucous membrane. × 6 dian 1. Lining membrane of cell in middle turbina 2. Bony core of turbinal. 3. Glands in submucou tissue on nasal aspect. 4. Mucous membrane nose.

PLATE I.—TO ILLUSTRATE MR J. S. FRASER'S PAPER ON THE HISTOLOGY OF NASAL ACCESSORY SINUS SUPPURATION.

Goetjes. The normal mucous membrane consists of two layers—superficial epithelium and submucous connective tissue, separated by a delicate basement membrane (Figs. 1 and 2). The surface layer is composed of ciliated cylindrical cells with a deeply placed nucleus: a varying amount of granules is also present in these cells. In some the amount is so large that the nucleus is hidden, in others the granules are scanty, and in others again they are absent and the cells appear as "ghosts." Hajek has shown that in the nasal mucous membrane this latter condition is associated with a discharge of mucus, and it is almost certain that the same thing holds good in the accessory sinuses. Between these superficial ciliated cells and the basement membrane there are one or two rows of cubical or spindle-shaped cells, the nuclei of which are large in proportion to the size of the cell. In normal cases the basement membrane is a very delicate structure, and is represented by a very fine homogeneous line just beneath the deepest layer of the epithelium. Goetjes states that under normal conditions a basement membrane is not present. The submucous tissue consists of several layers of elongated connective tissue cells with their long axes parallel to the surface of the mucous membrane. Eschweiler has divided this layer into two—a looser superficial and a denser deep layer—the latter forming the periosteum of the subjacent bony wall. It is in this latter that the blood-vessels run, while in the superficial stratum are occasionally found the coiled mucous glands which form such a prominent feature of the submucous tissue of the inferior and middle turbinal bodies. It is usual to find an aggregation of these glands in the neighbourhood of the ostium (Fig. 3). It should perhaps be pointed out that there is not the same necessity for a large number of mucous glands in the accessory sinuses as there is in the nose itself: the interchange is very slight between the air contained in the sinuses and that passing to and fro in the nasal cavities; the drainage of the sinuses would also be extremely difficult if a large quantity of mucous secretion were poured out in these cavities. Goetjes states that a certain amount of round cell infiltration is to be found in the normal submucous tissue of the sphenoidal sinus just as in the nasal mucous membrane. I have not been able to confirm this observation.

Changes in the Mucous Membrane in Acute and Chronic Inflammation.—Nineteen cases have been examined, distributed as follows: antral mucosa 9, ethmoidal 8, frontal 1, and sphenoidal 1; for 2 of the antral cases I am indebted to Dr Logan Turner.

*

Acute Inflammation.—Only one case of acute inflammation in the mucous membrane of a sinus was met with (Fig. 7) ; this was in the sphenoidal sinus mentioned above, which was obtained at the autopsy on a case of acute pneumonia. The mucous membrane was considerably thickened and deep red in colour ; the surface was velvety, and the sinus contained some thick muco pus—but no blood. On microscopical section the prominent feature was the extensive hæmorrhage in the submucous tissue and the engorgement of the vessels : small cell filtration was not so marked as in chronic cases, and the œdema was slight in amount ; the superficial epithelium was in places markedly disintegrated. Goetjes states that in acute inflammatory conditions the number of beaker cells and consequently the production of mucus is greatly increased ; finally, the superficial layer of cells desquamates, or the whole of the epithelial covering may be lost. In four cases of acute inflammation of the mucous membrane of the sphenoidal sinus Goetjes found marked round cell infiltration, most intense just under the epithelium, but also present in the deeper layers around the glands and vessels. Submucous hæmorrhages were present in three out of four cases. The round cells consisted mainly of lymphocytes, but eosinophiles phrynuclear and plasma cells and isolated phagocytes were also present.

Chronic Inflammation.—In the first place a more or less thick layer of catarrhal or purulent secretion is present on the surface (Figs. 14 and 15) ; it is important not to remove this layer during the process of preparing the specimen for examination, because, if it is present, we can exclude with certainty the fallacy of an artefact when talking about the condition of the superficial layer of cells. The secretion or exudation consists of mucous and leucocytes in various proportions, with occasionally epithelial cells.

Changes in the Superficial Layer.—It is remarkable how often this layer appears to be normal, although there are marked changes in the submucous tissue. Not infrequently, however, the number of ghost cells in the most superficial layer is very large, while in other cases this ciliated layer is absent in parts and only the spindle-shaped or cubical cells beneath are left. Occasionally the basement membrane is entirely denuded of epithelial covering (Fig. 10). In most of the specimens leucocytes may be seen making their way between the epithelial cells to the surface—there to mix with the mucous secretion and form the *muco*-purulent or purulent exudation characteristic of sinus catarrh or suppuration (Fig. 9).

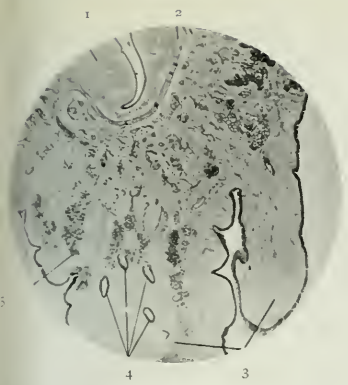


FIG. 5.—Early inflammatory œdema of middle turbinal (nasal polypus). × about 6 diam. 1 (Edema of submucous tissue of air-cell in middle turbinal. 2. Bone. 3. Pendulous masses of œdematous connective tissue (polypi). 4. Dilated gland ducts. 5. Mucous glands.

FIG. 6.—(Edema of middle turbinal in case of ethmoidal suppuration. The normal tissues are crowded towards the bone; the thickened lining membrane of the cell in the middle turbinal is well seen, and the small-cell infiltration of its mucous membrane. × 6 diam.

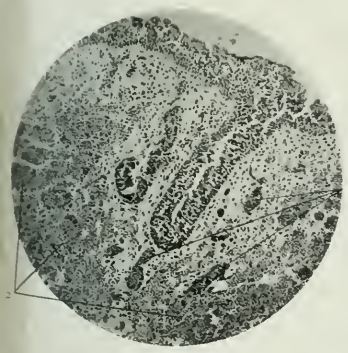


FIG. 7.—Mucous membrane from sphenoidal sinus showing acute inflammatory changes. × 50 diam. 1. Engorged blood-vessels. 2. Hæmorrhages. Notice disintegrated condition of surface epithelium.

FIG. 8.—Polypoid mucous membrane from floor of maxillary antrum. × 9 diam. 1. Apparently normal epithelium on surface. 2. Stalk of the polypus. 3. Large connective tissue spaces filled with serum.

PLATE II.—TO ILLUSTRATE MR J. S. FRASER'S PAPER ON THE HISTOLOGY OF NASAL ACCESSORY SINUS SUPPURATION.

In one and the same specimen mononuclear cells may be seen at one point between the epithelial cells and at another only polymorphonuclear cells ; eosinophiles may also occasionally be found in the same situation. To a smaller extent this process may be observed in the nasal mucosa in cases of chronic catarrh, but in this condition the leucocytes which have come through the epithelium on to the surface are diluted by the large quantity of mucous secretion poured out by the vast number of mucous glands in the nose, and consequently the discharge is only *muco*-purulent, whereas in the accessory sinuses the mucous secretion is scanty and the leucocytes at least equally numerous, so that the discharge is more definitely *purulent*. With regard to metaplasia of the epithelium, after going very carefully over all my specimens I found small areas in three of the nine antral cases (33 per cent.) in which the superficial cells approximated more closely to the squamous than to the cylindrical type (Fig. 9) ; Oppikofer found this condition present in 41 per cent. of antral cases. The question of the relationship of cholesteatoma of the accessory sinuses and middle ear to this metaplasia of the epithelium is not yet settled : so far a case of cholesteatoma has not been recorded in which there was no possibility of the epithelium coming in from a surface covered by squamous epithelium (mouth, skin, external auditory meatus), but cases have been described in which this metaplasia of the epithelium was regarded as the cause of the cholesteatoma formation. In none of my specimens did the loss of superficial tissue extend deeper than the basement membrane, so that nothing in the nature of ulceration was met with in the accessory sinuses. Goetjes states that it is not possible to distinguish acute from chronic inflammatory processes by the epithelial changes.

The *Basement Membrane*, which is extremely delicate and may apparently be absent in the normal accessory sinuses, is as a rule markedly thickened in cases of chronic suppuration.

Changes in the Submucous Tissue.—The thickness of the mucous membrane varies considerably in different parts, being greatest in the hollows and least over the convex surfaces : this variation depends on the amount of submucous tissue. In chronic inflammatory conditions this tissue is usually much increased in depth, the increase being mainly due to œdematous infiltration, though fibrous changes, vascular engorgement, and small cell infiltration no doubt play a part. Goetjes looks on sclerosis of the submucous tissue as an indication that cure had resulted in a case of chronic inflammation (Fig. 16). I agree with Oppikofer in thinking that

the degree of thickening of the submucous tissue is not a measure of the severity of the suppurative process, and that a slightly thickened mucous membrane may suppurate freely and cause the same symptoms as a sinus with enormously thickened polypoid mucosa. In fact I think that many cases of this latter type are not clinically or pathologically severe, and are more allied to conditions of simple nasal catarrah and polypus formation (compare Fig. 8 with Fig. 11).

The œdematous infiltration results in a widening of the delicate connective tissue network, so that large spaces are formed which are filled with serum : the process is in fact exactly similar to that found in the middle turbinal in cases of nasal polypus formation and in the inferior turbinal in so-called " chronic hypertrophic rhinitis." This polypoid character shows itself especially in the dependent parts of the sinuses, *e.g.* along the floor of the maxillary antrum : the condition is not difficult to explain, for we have only to remember that the mucous membrane accurately fitted the antrum when the submucous tissue was of normal thickness ; if now this tissue increases tenfold in depth, it follows that, if the epithelium on the surface is to remain of the same superficial area, it must become folded on itself—or, in other words, polypoid. As seen in section this tendency is naturally most marked in the dependent parts of the sinus (Fig. 8).

Small Cell Infiltration.—This varies markedly in situation, in degree, and in the character of the cells : in some cases there is marked œdema and marked small cell infiltration. Again, the infiltration may be confined to that part of the submucous layer which lies just under the basement membrane (Fig. 6), or it may be more or less evenly distributed over the whole thickness of this tissue, or it may be in places aggregated into lymph nodules. I have not observed it specially marked in the deepest layer of the submucosa, but such appearances have been reported by Oppikofer. In some cases the submucosa resembles embryonic connective tissue (Fig. 14). The great majority of the cells are lymphocytes : this is interesting, because cytological examination of the fluid washed out of the maxillary artrum and nose by puncture through the outer wall of the inferior meatus frequently shows the cellular elements to consist almost entirely of polymorphs. I do not pretend to be able to explain this discrepancy, but I think that it is important in these cases to use a stout hollow needle which can be fitted on to the barrel of an evacuating syringe, so that any discharge present in the antrum

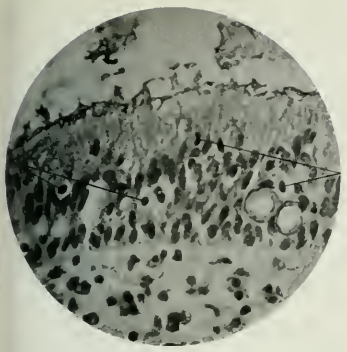


Fig. 9.—Superficial epithelium from case of chronic maxillary antral suppuration. × 1000 diam. 1. Leucocytes making their way to the surface through the epithelial layer. Notice the catarrhal exudation on surface.

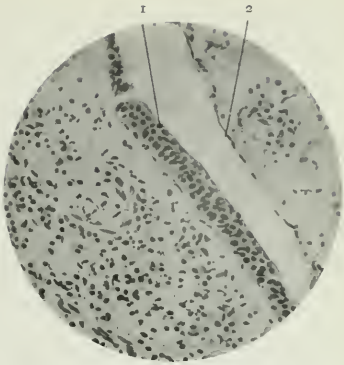
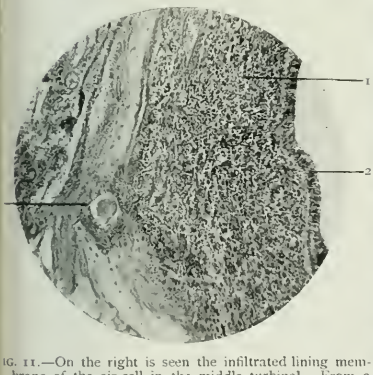


Fig. 10.—Mucous membrane from maxillary antrum from a case of chronic suppurative catarrh. × 300 diam. 1. Metaplasia of superficial epithelium—approach to squamous type. 2. Complete loss of epithelium—the basement membrane, however, remains.



ig. 11.—On the right is seen the infiltrated lining membrane of the air-cell in the middle turbinal. From a case of chronic ethmoidal suppuration. × 50 diam. 1. Small-cell infiltration is very dense, but œdema is slight. 2. Columnar epitbelium. 3. Small vein showing fibrous thickening of wall.

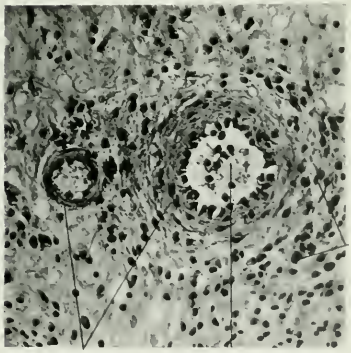


Fig. 12.—Chronic suppurative catarrh of the maxillary antrum. × 300 diam. 1. Small-cell infiltration of connective tissue. 2. Catarrhal secretion in gland duct. 3. Condensation of fibrous tissue around duct.

Plate III.—To Illustrate Mr J. S. Fraser's Paper on The Histology of Nasal Accessory Sinus Suppuration.

may be withdrawn into the syringe and examined without the risk of contamination by the mucopus and micro-organisms present in the nose.

Glands.—The gland-ducts in the submucous tissue may be dilated and filled with mucus, degenerated cells, and leucocytes: in one case I observed marked fibrous thickening round the ducts (Fig. 12). Cysts are occasionally found in the submucous tissue (Fig. 13); these are filled with homogeneous "colloid" material, and lined by cubical cells; they are of course due to blockage of the gland ducts, and are said to be more frequent in the antrum than in the frontal sinus: they are not diagnostic of chronic suppuration according to Oppikofer. Goetjes frequently observed cysts in the sphenoidal sinus mucosa.

Changes in the Vessels.—The great engorgement characteristic of acute inflammation is not present in chronic cases to the same extent, but still a certain amount of vascular dilatation is to be observed. Thickening of the vessel walls was observed in one of my cases, and Oppikofer has described hyaline changes in the walls in otherwise healthy (presumably non-syphilitic) people. I have observed one such case. The small cell infiltration is usually very marked round the vessels. Old hæmorrhages are not infrequently seen in the submucous tissue, and deposits of pigment are also found (Fig. 15).

Changes in the Bone.—In the cases of antral, sphenoidal, and frontal suppuration the bony wall of the sinus was not included in the tissue examined, but out of the eight ethmoidal cases osteoclasts were found in only one instance. Bone disease may be present in accessory sinus suppuration, but it is not an essential change, and is due to the more or less gradual spread of the inflammatory process from the surface through the mucous membrane. Grünwald found bone disease in 18 per cent. of antral cases, and in 60 per cent. of ethmoidal cases, and Oppikofer states that the bone often appears to be eroded with numerous lacunæ-like depressions, that lamellar formation is irregular, and bone cells are enlarged; he states that osteoclasts are very rare. I have observed them in one case of ethmoidal suppuration.

Micro-organisms.—I have not been able to find organisms in the tissues in a single case, although numerous sections of all the cases were examined after staining by Gram's method in addition to the ordinary stains. In the discharge lying on the surface, organisms could occasionally be seen. Dr Turner reports that Mr

Wade found organisms present in each of four antral cases, and in three of these the bacteria were present in the cells of the mucous membrane, while in one case there were small abscess cavities crowded with cocci in the submucous tissue. Lack expresses the view that there is no definite evidence at present to show that a particular organism or mixture of organisms is associated with a definite sort or degree of inflammation.

What is wanted, if we are ever to obtain useful knowledge as to the prognosis and method of treatment in a given case of sinus suppuration, is an extensive investigation into the subject: if we could, from a large series of cases, obtain a table giving the cause, duration, symptoms, rhinoscopic appearances, cytological and bacteriological examination of the discharge, along with an account of the histology of the mucous membrane if the case came to radical operation, we might be able to make a considerable advance.

Comparison of the Simple Inflammatory Processes in the Upper Respiratory Tract, including the Middle Ear Cleft.—In a paper published in the *Journal of Laryngology* (Aug. 1908) I showed that in nasal polypus formation and in inferior turbinal hypertrophy the condition was essentially one of chronic inflammatory œdema or serous exudation into the connective tissue spaces under the basement membrane: the amount of leucocytic infiltration is slight, while the superficial epithelium is, as a rule, practically normal. On the other hand, from the microscopical examination of the nasal mucosa in cases of ozœna, it is apparent that the change in the superficial epithelium is very marked—large tracts are converted into squamous epithelium, and keratinisation of the superficial cells is sometimes present; the glands are atrophied, and there is intense leucocytic infiltration of the submucous tissue; œdema on the other hand is absent. We find, however, not infrequently that the inferior turbinal shows typical atrophic changes, while the middle turbinal shows a polypoid condition—a fact which supports my view of the pathology of these conditions.

In the accessory sinuses similar changes may be met with—thus, two of my cases show œdema of the submucous tissue with intact epithelium and little or no leucocytic infiltration (Figs. 5 and 8). Kahn also remarks on the similarity of one of his cases to nasal polypus formation. On the other hand, many sinus cases that come to the radical operation show changes which closely correspond to such conditions as suppurative rhinitis or ozœna. In these cases we have dense leucocytic infiltration with marked

changes in the superficial epithelium (loss of ciliated layer, etc.). It is of course obvious that the accessory cavities are not, like the nose, subject to the drying influence of the air currents, and are not so liable to mixed infection; consequently we do not meet with the foul-smelling dry crusts in the accessory sinuses.

Catarrh of the mucous membrane of the accessory sinuses is much more common than is usually supposed. Oppikofer found that about half of his 94 *post-mortem* cases of sinus inflammation might be classified as *catarrh* and half as *suppuration*. All specialists are well acquainted with the type of patient who comes complaining of nasal discharge and obstruction, with perhaps a heavy feeling over the bridge of the nose or glabellar region. On examination the turbinals are found to be enlarged—the middle turbinal polypoid—and there is excess of mucopus in the nose. The antra fail to illuminate, though the frontal sinuses as a rule light up well. On proof puncture of the antra only a little mucus or flaky mucopus is washed out. The number of cases which fail to illuminate is too large to be accounted for by anatomical causes, such as thick bony wall. I do not, however, mean that accessory sinus catarrh is present in all cases of nasal catarrh, or that nasal suppuration is to be found in all cases of sinus suppuration. The position of the ostia of the accessory sinuses, while it renders infection of the mucosa of the sinus difficult, also tends to prevent the spontaneous cure of a severe sinus infection. The nasal mucosa on the other hand is frequently affected by catarrhal processes, but the drainage conditions in the nose are much more efficient than those in the accessory sinuses. In short, the nasal mucosa, though frequently infected, has a great chance of recovery, while the accessory sinuses, though not so exposed to simple or suppurative catarrhal processes, are less able to throw off these conditions once they have occurred.

Finally, although the number of my specimens of acute and chronic middle-ear inflammation is small—four acute and nine chronic cases—I think that it is possible to show that the changes here are similar to those in the nose and in the accessory sinuses: in other words, we have acute catarrh, chronic catarrh with œdematous swelling, acute and chronic suppurative processes. The difference between catarrhal and suppurative processes is only one of degree, and there are borderland cases. Otologists are all familiar with the type of acute otitis media which occurs in children with adenoids, where the tympanic membrane is slightly congested, cloudy, and bulging: such cases may go on to suppuration or they may not,

and, even if perforation does occur, there is only for a day or two the escape of a thick muco-purulent discharge in which the mucous element predominates. Such cases are on the borderland of what is called " catarrh " and what is called " suppuration," and from this mild type we have all grades of severity up to those malignant cases in which within a few days the tympanic membrane is almost entirely lost and bone disease occurs in the ossicles and in the walls of the middle-ear cleft—more especially in the mastoid cells, where the drainage is least efficient. Nager has confirmed the observations of Burckhardt-Merian and others that there is a parallel—if not indeed an etiological connection—between the severe nasal and pharyngeal affection of scarlet fever and the different forms of middle-ear suppuration. Nager has very seldom observed a severe scarlatinal diphtheria of the nose and pharynx without participation of the middle ear. Again, we know the type of case in which after signs of acute inflammation have been observed in the tympanic cavity, the membrana tympani and hearing power return to normal, but the mastoid inflammation continues, and an abscess forms. Neumann and Ruttin have shown that the diplococcus mucosus capsulatus is the organism present in many of these cases. Such cases appear to correspond to those of maxillary antral suppuration in which the nasal cavities present normal appearances. Finally, I venture to suggest that in the classification of acute and chronic simple inflammatory processes in the upper respiratory tract (including the middle-ear cleft) it would be well to consider the changes not only in the submucous tissue, as Eschweiler has proposed, but also in the epithelial covering. Under the heading of *Simple Catarrh* we might include cases with œdema of the submucosa with slight alteration of the superficial epithelium and only moderate leucocytic infiltration of the submucous tissue; under the heading of *Suppurative Catarrh* we might include four more or less distinct conditions, (1) cases with little or no œdema and only slight epithelial changes, but with marked leucocytic infiltration (Fig. 11), (2) cases similar to the above, except that the epithelium has undergone metamorphosis or is almost entirely absent over large areas, (3) cases with marked œdema and leucocytic infiltration, but with slight epithelial changes, (4) cases with marked œdema and leucocytic infiltration, but with extensive changes in the epithelial layer.

In conclusion I should like to express my thanks to the Royal College of Physicians (Edinburgh) for kindly allowing me to work

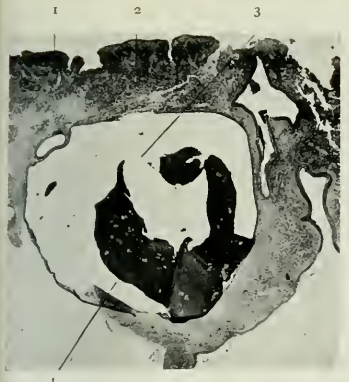


FIG. 13.—Mucosa of maxillary antrum from case of chronic suppurative catarrh. × about 20 diam. 1. Superficial epithelium. 2. Dense small-cell infiltration of submucosa. 3. Cystic space (lined by cuboidal cells). 4. Colloid secretion in the cyst.

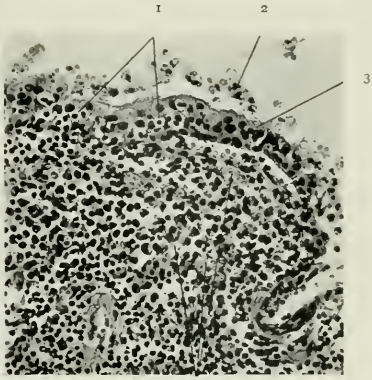


FIG. 14.—Mucous membrane of antrum from case of chronic suppuration. × about 300 diam. 1. Leucocytes passing through epithelial layer to the surface. 2. Purulent secretion. 3. Epithelium showing loss of ciliated layer. 4. Engorged blood-vessels.

FIG. 15.—Mucous membrane of maxillary antrum from case of chronic suppurative catarrh. × 60 diam. 1. Catarrhal secretion on surface. 2. Superficial epithelium. 3. Hæmorrhages in submucous tissue.

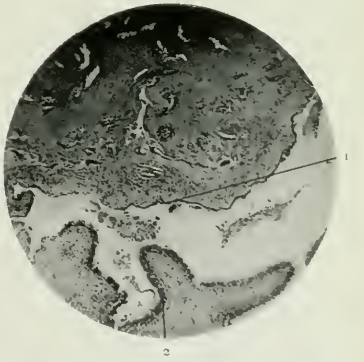


FIG. 16.—Mucous membrane of frontal sinus from case of rhinitis caseosa. × 50 diam. Shows fibrous change in submucous tissue. 1. Loss of superficial layers of epithelium. 2. Almost normal epithelium.

PLATE IV.—TO ILLUSTRATE MR J. S. FRASER'S PAPER ON THE HISTOLOGY OF NASAL ACCESSORY SINUS SUPPURATION.

in the Laboratory, to Dr James Ritchie for his advice and help in the preparation of this paper, and to the Carnegie Trustees for providing the photomicrographs.

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ANEURISM OF THE ANTERIOR CEREBRAL ARTERY, WITH UNUSUAL PROLONGATION OF LIFE AFTER RUPTURE: AUTOPSY.

(With Plates 22 and 23.)

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THE following case of rupture of an intracranial aneurism appears to us to be of sufficient interest from the clinical and pathological aspects to merit publication :—

A. R., single, 33 years of age, smith, was admitted to the Royal Infirmary on 18th April 1908 in a state of unconsciousness, which was said to have lasted about two hours. At 11.30 A.M. he had become suddenly ill, and the foreman, who had been called to see him, found him at his bench leaning forwards and supporting himself by his extended arms. His face was flushed, and beads of perspiration stood on his forehead. He had been ill for three or four minutes, was quite conscious, and said that he had had a similar attack one week before from which he had recovered in a few minutes, as he expected he would be able to do in this case. He complained of great pain in the head and back of the neck, and of great giddiness. With assistance he was able to walk out into the open air. He sat down for about ten minutes, and, feeling better, started to walk home in company with a boy. After he had gone about 300

yards, however, he was unable to proceed further, and the foreman, on being called by the boy, found him lying unconscious in the street, assisted him into a cab, and brought him immediately to the Royal Infirmary, which he reached in a state of complete unconsciousness.

When examined on admission, patient was lying on his back with his arms by his sides and his legs extended. The face was slightly congested, the eyelids half closed, and the palpebral apertures equal in size. The pupils were contracted, quite circular, and equal in size. They reacted to light only to a slight degree, if at all. Both conjunctivæ were quite insensitive to touch. The eyeballs were not prominent, and did not move at all. The mouth was shut. There was a small amount of frothy saliva between the lips. The lips were not livid, but were blown out with each expiration. The breathing was irregular both in rhythm and in depth of inspiration, but there was no stertor. Coarse rhonchi in the lungs masked the character of the breathing, but it was possible on auscultation to hear a slight systolic murmur at the mitral area. There was no abnormal percussion dulness, and no want of expansion of the chest, and, as far as could be ascertained from sounds made on respiration, no alteration of vocal fremitus or resonance.

The arms lay along the sides of the trunk, but there were frequent spasms in which they were forcibly extended at the elbow and drawn to the side of the chest, or even in front of it. The forearms were very forcibly pronated, so that the backs of the hands were directed towards each other. The wrists were flexed, and the fingers were fully flexed, causing the hand to be tightly clenched, the thumbs being strongly adducted against the index fingers.

During these tonic spasms the lower extremities were tonically extended, and there was slight extension at the ankle joints and inversion of the feet. The lower extremities were not affected during every seizure.

The spasms lasted a few seconds, were usually accompanied by a loud expiratory effort and puffing out of the cheeks, but the facial muscles were otherwise not involved.

The breathing was at the rate of about 30 per minute, and was quiet in the intervals between the spasms. The mouth was kept shut, and any attempt to open it was strongly resisted.

The head tended to rotate to the left side, and the chin was approximated to the left shoulder. The attitude of the patient after each spasm was almost exactly that represented by Pl. 22, Fig. 1.[1] The position of the arms during the spasm is very similar to that depicted in Fig. 175 in Vol. II. of Gower's "Diseases of the Nervous System," 2nd Edition, which represents the phase of tonic immobility or tetanism during the first or epileptoid period of hysteria major, and is reproduced from Richer's "Études Cliniques sur l'Hystero-Epilepsie ou Grand Hysterie." The shoulders were less raised and the contractions less intense, but the relative positions of the forearm and hands, and the clenched attitude of the fingers, were similar in both cases.

The reflexes in the lower extremities showed a slight exaggeration. There was ankle clonus on both sides, but both plantar reflexes were of the flexor type.

The axes of the two eyes were not parallel, there being a slight divergent strabismus.

The pulse was about 70 per minute, regular in rhythm, of good force and moderate expansion. The tension, as measured by Gaertner's tonometer, was 95 mm. Hg.

The temperature was 96·4.

It was ascertained from his relatives that, with the exception of the attack above referred to, his previous health had generally been good, but that there was a slight tendency to tuberculosis in his family history.

The condition remained more or less as above described from about half-past twelve until three o'clock. The respirations tended to assume the Cheyne-Stokes character. The temperature remained about 96·6; the patient still lay on his back, breathing noisily and rapidly. By 3.30 the pulse had risen to 100; the respirations were more definitely of a Cheyne-Stokes character, and at the height of the cycle the arms and legs tended to become rigid and the back to be arched. At that time the temperature, which had begun to rise, had reached 98·2°, and by four o'clock had registered 100·8°. There was free

[1] This is not a photograph of the patient himself, but is one of the case observed by one of us (A. B.), and described by Bruce and Drummond in the *Review of Neurol. and Psychiat.*, vol. ii., p. 737. It had been mislaid when that case was published.

perspiration on the face and over the front of the chest. The patient began to perform lateral and vertical chewing movements.

By 4.30 P.M. the temperature had reached 101·4°; the pulse was 110; respirations 45 per minute on an average, varying from 30 to 65 per minute according to the phase of the cycle at which it was counted. The Cheyne-Stokes character was then even more pronounced.

As the temperature was rising so rapidly and ominously, ice was applied to the trunk and limbs, with the result that the temperature fell from 101·6° to 97·6°, and the pulse from 120 to 90, the respirations remaining at 48 per minute. After the application of the ice, the spasms and chewing movements ceased. Loud rhonchi were heard over all the chest, obscuring the heart sounds.

The urine, which had been drawn off by catheter previous to the application of the ice, had a specific gravity of 1033, and showed a trace of albumen, but no other abnormal constituent.

Lumbar puncture was performed, and 25 c.c. of a brownish red fluid were drawn off under high pressure. This fluid was obviously composed of blood and cerebro-spinal fluid mixed, and, after standing in the test-tube, the solid elements began immediately to settle, and the precipitation was almost completed in two hours. On examination it was found that albumen was present in large amount. No sugar was present, but there was a distant though partial reduction with Fehling's reagent, a greenish colour with flaky precipitate being produced on heating. Blood was present, the guaiac test being positive. On microscopical examination the films showed:

(1) Large number of crenated red blood corpuscles; (2) a few polymorpho-nuclear leucocytes; (3) a few lymphocytes; and (4) a few large mononuclear cells.

The red blood corpuscles were the most striking corpuscular elements in the films. Many were perfect in shape and size, and many were reduced to the merest broken-down shell of an erythrocyte. All gradations between the two limits were found, very many of the corpuscles being beautifully crenated, although the fluid was microscopically examined as soon as it was obtained. The white cells present were polymorphs, lymphocytes, and large mononuclears: none of those which were phagocytic showed any organ-

isms in their interior. In some films there were some staphylococci (few) and a few rod-shaped organisms, but none were very definite, and none were intracellular. Their presence was probably due to accidental contamination. When the fluid was cultured, these organisms proved to be staphylococci (albus and aureus).

At 8.30 the temperature was still 98·8. The patient was perspiring freely. The pupils were moderately dilated, the right being greater than the left. Their outline was not quite circular. The jaws were firmly clenched.

At 9 P.M., the breathing was much quieter, and the spasms had ceased since eight o'clock, with the exception of the chewing movements and some movements of the tongue. The conjunctival reflexes had returned on both sides.

By 9.20 the pupils had begun to react to light, and the breathing had become more quiet and regular, altogether without stertor. This improvement in the condition remained fairly constant until eight o'clock on the following evening, the 19th, when the patient looked considerably improved, but about 9.30 P.M. the face became more congested and cyanosed, the perspiration stood in beads on his forehead, the temperature suddenly fell from 98° to 96.8°, and by 10 P.M. it had fallen as low as 95°. During the same time the respirations had increased from 38 to 48 per minute, and the pulse from 124 to 160, and at times it became so frequent that it was difficult to count it. The arms lay flaccid by the sides of the trunk, free from spasms or twitchings of any kind. The pupils were dilated, but were circular and equal. The contraction to light was practically gone, and the conjunctivæ were quite insensitive. The coma was now much deeper than on admission.

Hot water bottles were placed round the patient, and the pulse gradually improved in strength and slowed down to about 150. The respirations became deeper and less noisy than they had been, and the temperature began again to rise, becoming normal by 11 P.M. By midnight the immediate danger seemed to have passed, but the coma continued. Rhonchi, sonorous and sibilant, were heard all over the chest, and slight percussion dulness appeared at the bases posteriorly. Patient was fed with egg and milk by means of the nasal tube.

By 4 A.M. of the 20th, the patient had further improved. His temperature was 97·6°; respirations, 32; pulse, 130. The

pupils both reacted slightly to light, and were circular. The conjunctivæ were again sensitive.

A certain degree of improvement in the condition continued until 4.30 P.M., when there was a slight aggravation of the symptoms. By 8 P.M. it was noted that the lower extremities were now quite flaccid, and no deep reflexes could be obtained. The plantar reflex was difficult to elicit, but was of the flexor type on both sides. The upper extremities were quite free from spasms. The condition of the heart and lungs remained as before.

On the 21st it was noticed that the flaccidity of the lower extremities was less marked, and that while there were no actual spasms in the upper limbs, the tonicity of the muscles was slightly increased.

At 6 A.M. there was a rise of temperature to 101, which was met by the external application of ice and iced water. During the forenoon the comatose condition gradually became less deep, the eyes were opened, the eyeballs moved, and an impression was made upon his relatives during their visits that he seemed to recognise them. The upper and lower extremities showed some degree of tonicity, and there was a slight, feeble response to stimulation of any part. The deep reflexes again reappeared, and were somewhat exaggerated. The superficial reflexes were all present, the plantar reflex being still of the flexor type.

The improvement which had set in during this day was maintained. The patient appeared to be conscious. Although he was not able to speak, he followed with his eyes the movements of his relatives who came to see him. It was thought that he made a voluntary attempt to expectorate the mucus accumulating in his pharynx.

On the 22nd the plantar reflexes became extensor in character; the rhonchi disappeared from the lung, and the percussion dulness from the base. The systolic murmur could no longer be recognised in the mitral area. The urine passed by the patient retained practically the same character as before.

From the 23rd to the 28th of April the patient lay in the condition above described. There were no convulsions and no spasms of any kind. The temperature varied somewhat, and each time it rose above 101° F. towels with iced water were applied. They invariably reduced the temperature to about 99°. On the 23rd of April iced towels were used 9 times; on the 24th,

9 times; on the 25th, 26th, and 27th, 7 times; and on the 28th, 5 times, and in every instance the application was followed by a fall of temperature. On several occasions the patient so far recovered as to seemingly recognise his friends, but he never spoke, although he frequently appeared to be on the point of saying something. When anyone approached his bed, he looked at the visitor and quite consciously followed his or her movements round the bed, the movements of the eyeballs being well carried out. At other times he was less conscious, and lay on the bed taking absolutely no notice of anything or anybody.

By a second lumbar puncture, made on the 27th, 15 c.c. of fluid was obtained. It flowed freely and appeared essentially similar in character to that previously obtained, and microscopical and bacteriological examinations gave similar results.

Examination of the blood showed that the red blood corpuscles were between 4,300,000 and 4,500,000. Hæmoglobin remained steady at 80 per cent. White blood corpuscles were: on the 23rd, 14,000; on the 24th, 17,000; on the 26th, 19,000; on the 28th, 27,000. The differential count showed about: polymorphs, 70 per cent.; lymphocytes, 19 per cent.; mononuclears, 10 per cent.; eosinophiles, 1 per cent. The average blood pressure by Gaertner's tonometer remained at 95°.

On the 29th the condition became more grave; the limbs became quite flaccid; the eyelids remained incompletely closed, the divergent strabismus being present, but the eyes tended to show asynchronous movements. The pupils were moderately dilated, the right being larger than the left. They did not react to light. The conjunctivæ were insensitive to touch. The upper and lower limbs were flaccid; there were no reflexes to pinching. Rhonchi and crepitations were again heard all over the chest. The coma deepened, and the patient died quietly at 6 P.M. on the 29th, having lived twelve days after the initial onset of the symptoms.

Autopsy.—The autopsy revealed evidence of incompetence of the mitral and tricuspid valves of the heart. There were on the cusps of the mitral valve several large nodules of old endocarditis on the opposed surfaces. The lungs indicated a degree of congestion in the lower lobes, and the kidneys showed evidence of slight sub-acute nephritis.

Spinal Cord.—The dura mater was somewhat tense. Under

the arachnoid a thin layer of blood-stained fluid covered the cord throughout its whole length. A few small calcareous plates were found in the arachnoid in the lumbo-sacral region. When the layer of blood under the arachnoid was removed, the meninges had a rusty-red tinge. There was great tortuosity of the veins of the posterior surface of the cord. The consistence of the cord was slightly reduced in the dorsal and cervical regions; in the lumbar region it was fairly firm. On section of the cord, in the lumbar region, the anterior cornua were found to be somewhat congested. There were no hæmorrhages visible to the naked eye. On dividing the upper extremity of the cord from the medulla, as soon as the arachnoid was incised a large quantity of blood welled out from underneath this membrane through the foramen magnum.

Brain.—On opening the skull, and dividing the dura mater, the remains of the blood which had escaped from the foramen magnum was found in the sub-arachnoid space, the sulci, especially on the right side, containing a large quantity of blood-stained fluid, and the summits of the convolutions in the lower frontal, temporal and opercular regions being separated from the arachnoid by a thin layer of blood. Over the whole of the vertex minute bubbles of gas were found in the sub-arachnoid space. The arteries at the base of the brain were all normal, and the veins of the vertex were greatly engorged.

On making a longitudinal transverse section through the brain below the level of the corpus callosum, it was found that the two septa lucida, the fornix and almost the whole length of the corpus callosum had been ploughed through by a hæmorrhage which had evidently arisen from between the lower part of the two frontal lobes and had passed upwards and backwards, its progress above being arrested merely by the pia arachnoid covering the corpus callosum between the two hemispheres. Some of the blood had also entered the lateral ventricles, and had coagulated there without, however, either tearing up or even seriously compressing the basal ganglia. The blood did not appear to have passed backwards through the aqueduct of Sylvius into the fourth ventricle. The main mass of the extravasated blood had evidently found its way directly into the sub-arachnoid space, and had spread freely through this space along the lines of least resistance, filling up the sulci, the sub-

arachnoid cisterns, and raising the membrane from the convolutions, passing backwards, and, to some extent, seeking an entrance into the fourth ventricle from the foramen of Majendie, and also passing along the spinal cord under the arachnoid.

On making transverse sections through the basal half of the cerebrum, after the brain had been carefully hardened in a 7 per cent. solution of formalin, a firm clot of blood was found between the two frontal lobes. This had evidently been the starting-point of the great extravasation just described, for when a part of the clot was carefully washed away by a gentle stream of water, an aneurism on the left anterior cerebral artery was exposed (Pl. 22, Fig. 2). This aneurism was almost spherical in shape, and about the size of a small pea—5-6 mm. in diameter. It was carefully removed along with an adjacent portion of the brain cortex, and after some further fixation it was embedded in paraffin and cut into serial microscopical sections. From these it was ascertained that the aneurism was situated in the fork produced by the Y-shaped division of the anterior cerebral artery into two branches. It had formed on one of these branches just at the point of division (Pl. 23, Fig. 3). Beyond the limits of the aneurism, the main artery and its branches were almost completely normal in their structure, except at two points, which will be immediately referred to. There was no trace of any thickening or degeneration of any of the coats of the arteries, and, as already stated, the arteries at the base of the brain were almost normal.

The aneurism was seen to be composed of two parts: (1) a small primary sac (*b c*, Pl. 23, Figs. 3, 4, 5) formed by a local dilatation of the wall of the artery from which it arose; and (2) a somewhat larger secondary sac, or false aneurism (*d* in Figs. 4, 5, and 6), which had arisen by rupture from the preceding sac. Its walls were formed merely by the condensed tissue of the pia arachnoid, and its cavity was almost completely filled by mixed blood clot. On one side of this there was a further coagulum formed by blood which had escaped into the general mesh-work of the pia arachnoid (Figs. 5 and 6).

Microscopical examination of the wall of the aneurism indicated that the *initial weakening of the vessel which led to its formation was due to a localised defect in the muscular coat.* This was most clearly evident at a part of the wall at which

dilatation was just commencing, and where other changes had not been produced to complicate the picture, and the same condition was also observed in one or two small aneurismal dilatations of the other branch of the anterior cerebral artery at a little distance from the main division. The nature of the defect of the middle coat is shown in Fig. 3, and also in Figs. 7 and 8. At *c* in Fig. 3, and in Fig. 7, which is a more highly magnified view of the same part of the wall of the sac, it is seen that the middle coat is simply absent, and that the adventitia and the intima are in direct apposition. There is no indication of any pathological change in either the adventitia or the intima of such a nature as to have been capable of causing the disappearance of the media, and it is seen that at the margin of the sac the middle coat has become somewhat rapidly thinned or tapered off. Over the sac it is entirely absent until the opposite margin is reached, when it again begins to appear, and to increase in thickness in the same manner as at the opposite side. The adventitia in no way differs from the normal. The intima is slightly thickened by the proliferation of its endothelium, and by the splitting up of the elastic lamina into a series of layers which, on cross section, appear as tortuous fibrils (see Fig. 8).

Where the bulging of the sac has reached a higher degree, further changes in the intima have developed, which obscure the nature and meaning of the changes in the middle coat. The intima here (Fig. 3, *b*) has undergone an exceedingly high degree of thickening by proliferation of its endothelium. Its boundary from the remnants of the media, can be best determined in sections which have been stained for elastic tissue by Weigert's fuschin-resorcin method. It appears from these that at the two margins of the sac (*b*), the elastic lamina, split and tortuous, as already described, comes for a short distance into immediate contact with the adventitia, and then dips down between the thickened intima and the remaining laminæ of the muscular fibres of the middle coat. Even here the remaining portion of the middle coat is distinctly defective.

The wall of the more fully formed part of the primary sac showed a great and irregular thickening of the endothelium of the inner coat, with here and there small remnants of the elastic lamina and slight thickening of the adventitia, but practically

no indication of the presence of non-striped muscular tissue. At one part of the wall of the sac a small rupture had taken place and the blood had escaped into the pia arachnoid tissue, which it had driven before it and condensed into a firm layer sufficient to resist, in the first instance at all events, the further progress of the extravasated blood, which had to a large extent become coagulated within its sac. A small channel had, however, been left through which the blood had escaped into the loose-meshed sub-arachnoid tissue. A small part of this had coagulated, and is shown at *e*, Figs. 4 and 5.

It was found that on the branch *a'* of the anterior cerebral artery there were one or two points at which similar defects of the middle coat were associated with slight aneurismal dilatations. One of these is seen at *i*, Fig. 6. This, as in the previous case, was situated near a point of division of *a'*.

With regard to the ætiology of the condition, previous observers, such as Oppenheim (2), Beadles (3), etc., have drawn attention to the frequency with which aneurisms of the larger cerebral arteries arise from their points of division and from certain other sites of predilection, and to the relative frequency of the occurrence of such aneurisms in young individuals in whom there is no other evidence of arterial disease. The fact that in this case there was complete absence of anything to indicate a reason for the local disappearance of the middle coat, seems entirely to favour the view that the predisposing cause of the aneurism was a congenital defect of this coat.

From the clinical aspect, the case was of unusual interest in several respects: (1) The occurrence of a transitory attack of giddiness, with headache a week previous to the commencement of the fatal illness; (2) the sudden onset with unconsciousness associated with convulsive seizures of a special character without, in the first instance, any definite paralysis on either side of the body, but with a later temporary flaccidity of the muscles; (3) the large amount of blood-stained cerebro-spinal fluid obtained by lumbar puncture on two occasions; (4) the duration of life for twelve days after the first onset; and (5) the apparent partial recovery during the illness.

With regard to the first transitory attack of giddiness, it appears not improbable that this may have been associated with either the first development of the secondary sac or with a slight

rupture of this sac and the escape of a limited quantity of blood into the pia arachnoid. If so the quantity that had escaped must have been comparatively small, for it did not impair the patient's faculties in any way.

The clinical history seems to indicate that there must have been at least three other occasions on which hæmorrhages had occurred, namely, on the date of admission, on the following day, and on the day preceding death, on all of which there was a marked aggravation of the symptoms, with signs of increased intracranial pressure.

The convulsive seizures so exactly simulated those observed by one of us (A. B.), and recorded in this *Review* (*loc. cit.*), as to suggest a similar cause. The patient's condition after the sudden onset seemed to indicate the probable existence of a hæmorrhage into the third and lateral ventricles, but a hæmorrhage which did not destroy the internal capsule on either side, as there was neither hemiplegia nor diplegia at first. In this case, therefore, it could not have been due to the rupture of any of the vessels in the basal ganglia.

The amount of blood removed by lumbar puncture was so much greater than that met with in ordinary cases of hæmorrhage into the ventricles as to suggest that the blood had descended into the spinal canal either under the dura or under the arachnoid. The presence of this large quantity of blood-stained fluid on lumbar puncture and the nature of the convulsions, as well as the initial absence of hemiplegia or diplegia, or of any of the symptoms of cerebral hæmorrhage, suggested during life the possibility of a rupture of an intercranial aneurism.

The duration of life after the onset was very remarkable, as in hæmorrhage into the ventricles such a duration is recorded only in a very small number of cases. Sanders (4), in his valuable work on ventricular hæmorrhage, has found that in about 65·7 per cent. of the recorded cases death has occurred within twenty-four hours, and in the great majority of these in less than twelve hours, rapid death being the rule and delayed death the exception. He refers to three cases in which death was deferred until the twelfth day, one in which it did not occur until the eighteenth day, and one in which life was prolonged for a month. In none of these cases was there any association with aneurism.

FIG. 2.

FIG. 1.

Sanders is inclined to explain the apparent improvement which appears to be by no means infrequent in cases of hæmorrhage into the ventricles, by the fact that the brain has become accustomed to the pressure, and that its function has thus become partially or completely restored. The improvement varies from a very slight degree to perfect restoration of consciousness. As a rule it is merely transitory and is followed by a relapse ; it may be from a second hæmorrhage. In one case there was in all probability a succession of slighter or more severe hæmorrhages from the aneurism, to the pressure of some of which the brain had become partially accustomed by the displacement of cerebro-spinal fluid.

The microscopical investigation of this case was made in the Laboratory of the Royal College of Physicians.

Description of Figures.

Plate 22.

Fig. 1.—To show attitude of wrists and hands immediately after a fit. Photograph of case recorded by Bruce and Drummond (*Rev. Neurol. and Psychiat.*, Vol. ii., p. 737), and reproduced to show the similar attitude in present case.

Fig. 2.—Frontal section through anterior part of brain of A. R., showing the basal halves of both hemispheres separated by blood-clot (black), in the anterior part of which, and to the right side, is seen the aneurism as a paler globular mass.

Plate 23.

a' and a'', branches of anterior cerebral artery ; b and c, opposite sides of aneurism ; d, secondary (so-called false) aneurism followed by rupture of the primary aneurism b, c ; e, hæmorrhage into tissue of pia arachnoid from secondary sac ; i, i', intima of wall of vessel ; m, media.

Fig. 3.—Shows the aneurism b, c, formed on one branch (a') of the anterior cerebral artery at the point of division into two branches, a' and a''.

At b, the intima has undergone great thickening, while the media has become thinned.

At c, the middle coat has entirely disappeared (see Fig. 7).

Figs. 4 and 5.—Show the aneurism b, c, lying between the now completely separated branches a', a'' of the anterior cerebral artery. By the rupture of the wall of b, c at its power part, a second sac has been formed. It is filled with partly-organised thrombus, and its walls are formed by condensed tissue of pia arachnoid.

At e, blood has escaped from the secondary sac.

Fig. 6.—d, The secondary aneurism, with thin walls, formed merely of condensed pia arachnoid tissue.

e, Hæmorrhage from the secondary sac into the pia arachnoid.

a' and *a''*, Branches of anterior cerebral artery.

a''', A small aneurism, with absence of middle coat.

Fig. 7.—(H. P. Zeiss Planar.) Part of wall of aneurism (*c* in Fig. 3), showing how the media, *m*, *m*, becomes rapidly thinned, and at *i* has completely disappeared, leaving merely the slightly thickened intima *i*.

Fig. 8.—(H. P. Zeiss Planar.) Section stained with Weigert's (resorcin fuchsin) stain for elastic tissue.

m, Media, terminated suddenly at the bend in the wall of the vessel at *i*, where the intima is alone present.

i', Intima thickened, and, when examined with hand lens, seen to contain numerous tortuous fibrillæ of elastic tissue.

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The Influence of Meteorological and other Conditions upon the Distribution of Bacteria in Urban Atmospheres.

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THE INFLUENCE OF METEOROLOGICAL AND OTHER CONDITIONS UPON THE DISTRIBUTION OF BACTERIA IN URBAN ATMOSPHERES.

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At the commencement of this research, in October 1905, the presence of micro-organisms in the atmosphere had already been made the subject of numerous observations which did not appear to need repetition. Accordingly, the following experiments were directed to special points in the distribution of bacteria in the open air. Urban atmosphere was selected for investigation, and attention was directed to elucidate the relationship, if any, which exists between certain meteorological conditions and the bacterial contents of air. In the course of the inquiry it became obvious that while particular meteorological conditions, such as rain and wind, exert a notable influence upon the micro-organisms obtainable from the air, there are other conditions not meteorological which are of great importance in their effect on the bacterial flora of urban atmospheres. Amongst these other conditions are density of population, the presence of special sources of pollution, *e.g.*, sewage-farms, refuse heaps, &c., proximity to the sea, to agricultural manure land, to lines of traffic, or to the habitations of animals.

A description of the methods employed in the research will precede the statement of the results as observed under different meteorological conditions. Emphasis will then be laid upon special conditions which influence bacteria in the air. The vagaries of our climate add largely to the difficulty of observation, since so seldom is it possible to repeat an experiment under exactly similar conditions as to meteorological phenomena. Comparative observations were therefore extremely rare in which temperature, humidity, pressure and wind direction were all the same. When, however, only one or two of the factors varied some useful conclusions might be drawn. Instead of stating my experiments in the form of a series of tables showing for each experiment all the meteorological conditions of the day, I have preferred to discuss the results with illustrative examples taken from the series. In this way

[1] This research has been accomplished by the aid of a grant from the Carnegie Trust, for which I desire here to offer my sincere acknowledgments to the Trustees.

I trust to make my contribution more readable, even though it may not show all the work which was done. Altogether, I conducted 332 experiments in different parts of Edinburgh.

It may be well to say at the outset that no method of observation is available which will show the total number of organisms present in the air. Each method shows only the number of organisms capable of development under the particular conditions employed. In a given quantity of air a certain number of organisms may be found which will develop aerobically at 37° C., in the same air the number developing aerobically at 15° C. is different, whilst under anaerobic conditions yet another number may be counted. The sum of all these counts will not represent the number of organisms present in the air owing to the facultative qualities of certain varieties of bacteria. For comparative purposes it is essential that the procedures adopted throughout should be identical. No absolute standard has yet been approved, but as the greater number of atmospheric organisms will grow on a gelatine medium, under aerobic conditions, at a temperature of 15°-20° C., this method of culture has been that adopted by most observers. So that when air is stated to have contained so many organisms per litre, one understands that the number given is that of the organisms present and revealed by this method of aerobic growth on jelly at room temperature. In addition to this method I have, in certain observations, also estimated the number of organisms capable of growth at 37° C.

Methods.—The methods employed to estimate the number of organisms present in the air may be divided into two main sections, methods of collection by gravitation and by filtration.

(*A*) *Gravitation.*—In this method the organisms in the air were allowed to deposit themselves upon the exposed surface of the culture medium. The deposit, while largely due to gravitation, especially on calm days, was also influenced by wind currents. It was not thought advisable to protect the medium from wind as air currents are natural, and it was desired to make the observations under as natural conditions as possible. An open situation was generally chosen so that the plates should not be exposed to exceptional deposits from trees, roofs, &c. The culture media were either solid or liquid, and were exposed in shallow Petri dishes, for varying lengths of time. The solid media commonly

used were agar and jelly, but occasionally an albuminous medium made of egg was utilised. These media were incubated under aerobic conditions; the agar and egg media at 37° C., and the jelly at 15° C. The colonies growing on these media were examined from day to day, their number counted, and their characters determined by their staining reactions, and by sub-cultures in various media. In order to compare one quantitative observation with another which might differ in the duration of exposure, the results in each experiment were calculated as the number of organisms deposited per square foot of surface per minute of exposure. The area of the plate exposed was calculated from the diameter of the plate by the formula for the area of a circle, viz., $D^2 \times \cdot 7854$. It approximated to one-fifteenth of a square foot, so that in estimating the number of organisms per square foot per minute it was only necessary to multiply the number of colonies on each plate by fifteen and to divide by the number of minutes of exposure. The duration of exposure varied with the season of the year, the temperature, the locality under observation, and the character of the medium. Thus, shorter exposures were given in summer than in winter, on warm days than on cold days, in the slums than in the suburbs, and of jelly than of agar. It was found that agar could safely be exposed for longer periods than jelly, without overcrowding the plate, except in slum areas. The organisms which develop at 37° C. are not so numerous as those which grow at 15° C. On the other hand, in crowded neighbourhoods the agar plates are apt to show "spreaders," or organisms which, instead of growing at 37° C. as isolated colonies, spread in 24 to 48 hours over a large part or the whole of a plate, and thus obscure an accurate count. With a jelly plate a few minutes' exposure is generally sufficient.

The liquid media used were one per cent. peptone broth and glucose taurocholate broth. These media were exposed in Petri dishes, just like the solid media, but for much longer periods, the usual duration of exposure being one hour. With a little practice, it was found possible to transfer the liquid after exposure to sterile tubes, and these tubes were incubated anaerobically at 37° C. The quantity of liquid exposed was 10 c.c., and these broth-plate experiments were designed to test the air under anaerobic conditions of culture. A long exposure is necessary, as, presumably, anaerobes will be present in air only in relatively small numbers.

(*B*) *Filtration.*—There are various methods of filtration available for filtering air, and various substances may be used for the purpose. The method selected was that of Petri, and the substance used was fine sand. The method of aspirating the air through the sand is by the displacement of water. If the observations are conducted in or near the laboratory premises, or always in one place, the water receptacle may be a large vessel, holding perhaps 50 litres, placed on a stand, and having a tap at the bottom by which the water can be run off to any convenient drain or gutter. The vessel must be air-tight and well corked, the cork pierced by a glass tube which can be connected by rubber tubing with the sand filter. This is convenient, since it is only necessary to attach the full water cistern to the sand filter, and to set the tap so that the 50 litres of water flow off slowly, drawing an equal quantity of air through the sand. It is essential that the rate of flow should be very moderate, or the filtration is imperfect. Such a water cistern is too heavy for convenient transport, so that for work in different localities a substitute had to be devised. This was found in a portable camera-stand, and two bottles, each capable of holding fully 2 litres. The bottles were graduated, so that exact measurement of the aspirated air was possible. By alternate transposition of the bottles, water, to the quantity of 2 litres each time, could be passed from one bottle to the other. The upper bottle was placed on the camera-stand, and also connected to the sand tube above and to the lower bottle at the ground level. The arrangement worked well in practice, and it was only necessary to carry 2 litres of water in order to examine the air in places where no water supply was easily available. At the conclusion of the aspiration the sand tube was wrapped in sterile filter paper, and taken to the laboratory. The sand was then washed in sterile distilled water, the sand from each chamber being transferred to a separate flask containing 25 c.c. of sterile distilled water. The sand was washed in the flasks by gentle shaking for a period of several minutes. Then, with sterile graduated pipettes, portions of the water were transferred to sterile Petri dishes, and culture-plates prepared by the addition of liquid agar or jelly. The quantity of water used for each plate varied from 1 to 0·1 c.c.

The plates, after being set, were incubated, the agar at 37° C., and the jelly at 15° C. The colonies were counted at the end of 3 days in the case of agar, and 7 days in the case of jelly.

By calculation, the total number of organisms present in each flask, *i.e.*, in each chamber of sand, was ascertained, and the sum of both chambers gave the number of organisms in the whole quantity of air submitted to examination. The results might then be stated as so many organisms per litre or per cubic metre.

A.—Gravitation Observations.

Meteorological Conditions.—(*a*) *Temperature.*—It is a somewhat trite observation that the number of organisms in the air varies annually with the mean temperature. Their number is small in winter, increases as the spring advances, reaches the maximum in summer, and diminishes again in autumn. The curve of mean temperature has a general correspondence with the curve of organismal incidence in the atmosphere. In the year under observation this was exemplified, but on discriminative analysis it appears that the influence of temperature *per se* may easily be over-estimated. The warmer seasons coincide also with the dusty seasons, when the purifying influence of rain is least, and since the germs do not find in air itself sufficient means of subsistence and life, and probably do not multiply to a large extent in the air, their larger number in summer time is more an accidental coincidence with, rather than a result of, the greater warmth of the air. While noting, therefore, this general coincidence, one does not attach much importance to temperature alone. Indeed, it would be easy to show that in urban surroundings it often happens that the number of organisms on a day of moderately high mean temperature may be less than on a day of lower mean temperature. For example, in the laboratory yard, on October 9, 1905, the mean temperature of the day being 53° F., the number of organisms deposited per square foot per minute was 172, whilst on the 20th of the same month, the mean temperature of the day being 36° F., the number similarly deposited was 342. These numbers refer to organisms developing on jelly at room temperature. There was no rain on either day, but in each case there had been rain two days before. The direction of the wind and the height of the barometer on the two days were not markedly different, but the relative humidity was higher on the earlier date.

To take another instance in a more open situation, one may

compare the numbers found in Roseburn Park. On February 7, 1906, with a mean temperature of 40° F., the organisms amounted to 126 per square foot per minute, whereas on the 30th May, with a mean temperature of 52° F., the numbers were only 75. On these dates the wind was in the same direction, and the relative humidity was not very different, though somewhat higher, on the later date. These instances could be largely increased if no allowance were made for the variance of other conditions than temperature. It would be absurd to lay much stress on individual observations; these are only adduced to show that the correlation between temperature and abundance of organisms is not invariable. In the same park in January 1906, on two days, each with a mean temperature of 39° F., the organisms numbered 24 and 36 respectively. If these observations be compared with the May numbers, it will be seen that the general rule of more germs in warmer weather is upheld.

(*b*) *Pressure.*—The influence of atmospheric pressure on the bacterial content of the air has not been so well defined as that of some other meteorological conditions. Has it any influence at all? and, if so, in what direction? It may be suggested that a high barometric pressure might increase the deposit of organisms from the air. But high pressure means dry air, and if organisms are more plentiful in dry air than in moist air, this might account for any apparent increase of deposit attributable to high pressure. It appears that the influence of barometric pressure in itself is very slight. It is difficult to get two observations in which the only variant is the pressure; but I can quote two in which the pressure appeared to be the chief difference, while the number of organisms was approximately the same. Plates exposed on the Calton Hill on February 7th and 14th, 1906, showed respectively 153 and 141 organisms deposited per square foot per minute. On both occasions the wind was south-west, the relative humidity 82, and there had been no rain prior to the experiment. The mean temperature was 40° F. on the first day and 33° F. on the second occasion. The pressure, however, on the two days was very different, being 30·2 on the first day and only 29·4 on the second.

In general a high barometer means a greater number of organisms. In West Princes Street Gardens, on the open bank beneath the Castle Esplanade, two observations were made on December 15th, 1905, and January 26th, 1906. The mean tempera-

ture was 46° and 48° respectively, the relative humidity 90 and 85 respectively, the barometric pressure 30·2 and 29·7 respectively, and the number of organisms 198 and 102 respectively. The wind on both days was west-south-west, and the difference in the numbers of organisms might be attributed to the high pressure on the day of greater abundance. Both days were fine, but it is possible that the difference may have been produced by the absence of rain for several days prior to the first experiment and the occurrence of showers on days preceding the second observation.

(*c*) *Rainfall.*—The purification of the air by rain is one of the most salient features of the bacteriological aspect of air. The organisms are washed out of the air into the soil, and the number in the air is thus greatly reduced. The effects of rain may be considered as immediate and remote. To illustrate the immediate effect of rain, observations were conducted before and after heavy rain. There was a certain amount of luck in the ability to make these comparative observations. It happened on three occasions that a test of the air had been made on a fine morning, that heavy rain followed shortly afterwards, and that a further observation was made in the afternoon. Though the number of these occasions were few, the results suggest that the extent of purification of air by rain corresponds in direct ratio with the duration and heaviness of the rainfall. The following table shows the numbers observed before and after rain and the average of all exposures in the same locality:—

Locality.	Character of Rainfall.	Number of Organisms per Square Foot per Minute. Incubation, 15° C.		Average Number for Locality.
		Before.	After.	
Laboratory Yard .	Heavy, 4 hours	162	28	182
„	„ 2 hours	172	66	182
Blackford Hill .	Light showers	67	46	72

Thus 4 hours' heavy rain reduced the organisms in the air by about 80 per cent., while a few days later 2 hours' heavy rain only effected a reduction of 60 per cent., the place of observation being in each case the same. In the case of light rain the reduction only amounted to 30 per cent.

In order to show what one may call the more remote effect of rain the following method is adopted. Here it is impossible to compare observations immediately before and after rain since the rainy period may have extended over several days, and the other conditions were often entirely different before and after the rainy period. So in this case I have compared the numbers of organisms deposited from the air after a rainy period with the average numbers deposited from the air of the same locality in all the observations made. Such a comparison can only be approximately accurate, but the results serve to show that rain prolonged over several days and varying in its heaviness produces a marked diminution in the number of organisms capable of deposition from the air.

Locality.	Character and Duration of Rainfall.	Number of Organisms per Square Foot per Minute. Incubation, 15° C.	Average of Locality.
Laboratory Yard	Heavy at times during week	117	182
Inverleith Park	Some rain on 5 previous days	27	82
West Princes St. Gardens	Much rain 2 weeks	30	132
Grassmarket	Rain 3 previous days	261	751
Grange Road	,, ,,	63	128
Colinton Road	,, ,,	18	30
Blackford Hill	Rain 7 ,,	15	72
Inverleith Park	,, ,,	33	82
King's Park	,, ,,	20	125
Craigentinny	Heavy rain several days	75	373
Calton Hill	,, 3 days	54	211
West Princes St. Gardens	,, several days	42	132
Seafield Road	Rain 7 previous days	15	263
Calton Hill	,, ,,	15	211
Craigentinny	Heavy rain previous day	75	373
Saughton Park	Rain previous days	24	93

(*d*) *Humidity.*—The relative humidity of the atmosphere in itself is not constantly associated with any direct variation of the number of organisms deposited from the air. The general rule that a dry and dusty air yields more organisms than a damp and clear air suggests a correspondence between a low humidity and

an increase in the number of organisms in the air. In the laboratory yard the average number of organisms deposited per square foot per minute was higher when the relative humidity was low. When the relative humidity was under 70 per cent. the average of 5 observations was 214; when the relative humidity was between 70 and 80 per cent. the average of 3 observations was 144; and when the relative humidity was over 80 per cent. the average of 6 observations was 131. While this is so, there are other conditions upon which the number appears to depend more than upon the relative humidity. For instance, upon four occasions on Blackford Hill in March, April, May and June 1906, the number of organisms deposited per square foot per minute was identical, viz., 60. Yet on these four occasions the relative humidity was 98, 66, 79, and 78 per cent. respectively, while the general direction of the wind and the barometric pressure were practically the same. The mean temperature certainly varied from 25° to 51°, and the similarity in numbers is probably due to the direction of the wind and the incidence of the rainfall.

(*c*) *Dust.*—Organisms in the air are frequently carried about on dust particles, and it would be interesting to show that there is a numerical relation between the dust particles and the organisms deposited at the same time and place. There were, however, considerable difficulties in the attainment of comparative observations. I am much indebted to Mr. John Aitken, F.R.S., for his kind advice and assistance in the matter. His perfect apparatus for the demonstration of dust particles in the air is designed to show extremely small particles, and was too fine for the purpose in view. I therefore used a glass surface which could be exposed alongside the culture-plate. It fitted inside a Petri dish, and was exposed just in the same way as the plate. The graduated portion of the surface measured 5 cm. by 2·4 cm., divided into 300 squares. The surface might be lightly smeared with glycerine or egg albumin to cause the dust to adhere, but later I found that this was not necessary if sufficient care were taken in transporting the dish into which the glass accurately fitted. The dust particles deposited were counted by the aid of the low power of the microscope ($\times 70$) in at least 30 squares. Estimation was then made of the particles per square foot per minute, and this number was then compared with the number of organisms deposited on the same area in the

same time and at the same place. The method does not admit of absolute accuracy; even during the counting with the microscope fresh particles may be deposited from the air, but the results serve to indicate a general tendency. If the number of organisms is high, the count of dust particles is also high. The very high numbers of both found on the Queensferry Road are due to the fact that the count was made immediately after the passage of a motor car, and to show definitely the effect of rapid traffic.

Dust and Micro-Organisms.

Locality.	Dust Particles deposited per Square Foot per Minute. (x 70.)	Organisms deposited per Square Foot per Minute. Incubation 15° C.
*Queensferry Road . .	889,053	1860
**Craigentinny . . .	402,257	185
*Princes Street Gardens .	314,856	232
*Grange Road . . .	273,126	130
*Calton Hill . . .	201,593	246
*West Meadows . . .	170,007	145
†Saughton Park . . .	54,904	70
†King's Park . . .	23,225	20
‡Ravelston Road . .	13,294	27
†Blackford Hill . .	10,838	30

(*f*) *Wind.*—Apart from rain, already dealt with, and from slum areas and polluted soils, to be referred to later, the influence of wind upon bacterial deposition from urban atmospheres has been the most prominent feature of the experiments. Over and over again it has occurred that experiments to windward and leeward of the town have disclosed an excess of organisms to leeward over those deposited to windward. It appears that in Edinburgh at all events this is true, independent of the direction of the wind. Further, not only the quantity but the quality of the organisms is changed. On the windward side there appear generally harmless organisms, while to leeward appear organisms of putrefaction and of sewage origin. The subsequent tables show the direction of the wind and the localities in which

* Localities near centres of traffic.
† Suburban public parks.
‡ Quiet suburban road.
°° Sewage farm.

simultaneous observations were made. The first of these tabular statements shows the number of organisms deposited per square foot per minute and capable of growing on an agar-plate when incubated at 37° C. Its importance is two-fold. It shows how the direction of the wind appears to influence the locality in which the greater number of organisms will be deposited. A fresh wind coming fairly clean into the town sweeps out the organisms, and larger numbers are deposited to leeward. The table also shows that to windward of a town the air does not contain many organisms capable of growth at blood temperature—a characteristic attribute of organisms pathogenic to human beings. In the increased number of these organisms found to leeward of a town there may be included examples of organisms capable of producing illness, and derived from the conditions of town life. The observation sites were so chosen that the wind to leeward had passed over the whole or a large part of the city.

TABLE SHOWING INFLUENCE OF WIND.

Organisms per square foot per minute. Incubation temperature, 37° C.

Direction of Wind.	Windward.		Leeward.	
	Locality.	Number.	Locality.	Number.
East . .	King's Park	4	Saughton	18
„ . .	„	6	„	24
West . .	Saughton	18	King's Park	45
South-West	Calton Hill	30	Seafield Road	42
East . .	Seafield Road	9	Calton Hill	18
„ . .	„	18	„	24
West . .	Colinton Road	5	King's Park	15
South-West	Roseburn Park	5	Seafield Road	36
North-West	Ravelston Road	17	King's Park	71
„ .	„	1	„	30
„ .	Inverleith Park	3	Blackford Hill	6

The next table shows the comparative numbers of organisms, deposited per square foot per minute, and capable of growth at 15° C., in windward and leeward localities. While not perhaps of the same possible clinical significance as the preceding table, it

shows the numbers of germs present in the atmosphere of different localities under certain wind conditions and the influence of wind on their dispersal.

Table Showing Influence of Wind.

Organisms per square foot per minute. Incubation temperature, 15° C.

Direction of Wind.	Windward.		Leeward.	
	Locality.	Number.	Locality.	Number.
East . .	King's Park	11	Ravelston Road	27
North-West	Ravelston Road	16	King's Park	42
South-East .	King's Park	20	Saughton Park	50
West . .	Saughton Park	46	King's Park	120
South . .	Blackford Hill	15	Inverleith Park	33
North . .	Inverleith Park	27	Blackford Hill	60
North-West	Roseburn Park	75	,,	105
South-West	Blackford Hill	60	Calton Hill	291
West . .	Colinton Road	42	King's Park	105
South-West	,,	18	Seafield Road	156
West . .	Roseburn Park	24	Regent Terrace	48
West . .	,,	36	Seafield Road	123

Locality.—There is a great difference between the numbers of air organisms present in the suburbs and in the centre of a large city. Densely populated districts contain many more atmospheric organisms than the more sparsely populated quarters. Taking the average number of organisms deposited per square foot per minute and capable of development at 15° C. as the basis of comparison, it is possible to compare the centre and the suburbs of Edinburgh with the following results:—By the average number of organisms, I mean the average of the observations made by me in the different localities during the year, and these numbers must not be taken as expressing the true average of the individual localities for the whole year. The average of 114 observations in the central districts, including such localities as the Cowgate, Grassmarket, Castle, West Princes Street Gardens, &c., was 269, while the average of 96 observations in the suburban districts, including Colinton Road, Blackford Hill, King's Park, Inverleith Park, Ravelston, &c., was only 76.

Seldom, indeed, were the plates exposed to air found sterile thereafter. But at Ravelston Road, near Murrayfield Golf Course, on a fine day near the end of November, an agar-plate was exposed for ten minutes and there fell on it no organisms which were capable of growth on agar at 37° C.—the temperature at which the plate was incubated. The jelly-plates exposed alongside of the agar-plate showed a few organisms capable of growth at 15° C. A similar observation was recorded one day in February 1906, on Blackford Hill.

In the proximity of slums the numbers observed are much larger than those mentioned above. In the Pleasance the average of 4 observations was 949, while in the Grassmarket the average of 10 observations was 751. These numbers contrast strongly with those observed in less crowded districts, *e.g.*, in Princes Street Gardens the average of 18 observations was only 132, notwithstanding the influence of the adjacent railway. Smaller numbers were found in the suburbs, *e.g.*, in Craiglea Drive only 24, at Murrayfield 20, and at Colinton Road 18.

The provision of open spaces, and the prevention of overcrowding of the population on an urban site, should have excellent influence in maintaining the purity of the air.

In investigating the question of locality, no results were more striking than those noticed in the Craigentinny district. The sewage farm there has a notable influence not only upon the numbers but upon the character of the organisms found in the adjacent air. The situation being open ground and with few dwellings, the organisms might be expected, in the absence of the sewage, to number about the same as in other suburban districts of the town. But actual observation showed that the numbers more closely approximate to those found in the Old Town. Forty observations on the Sewage Farm yielded an average of 332 organisms. Organisms like B. coli communis and other germs common in sewage appeared in the plates. Typical B. coli was isolated on 14 occasions, and atypical forms of the same organism were sometimes present. The jelly-plates were frequently offensive and apt to liquefy. Organisms of the Proteus, Fluorescens, and Mesentericus groups were chiefly responsible for the odour and liquefaction. On almost every occasion organisms belonging to one or other of these groups were recognised in the plates. Moreover, streptococci occurred with greater frequency in this district than in

ordinary air, and they were not of the type of the common air streptococcus but rather resembled fæcal streptococci. There can be no doubt that the presence of the sewage farm profoundly modifies the flora of the adjacent atmosphere.

Some interesting observations were made by the exposure of plates at Seafield Beach. When the tide was full up, the day fine, and the wind blowing off the sea, the plates showed very few organisms. On the other hand, an exposure in the same place, with the tide full up, and the wind blowing off the sea, but bringing up with it a thick easterly haar or wet fog, resulted in the jelly-plates being crowded with liquefying organisms. In this particular situation the state of the tide was found to be important, because when the tide was out and the wind blowing off the sea the plates were apt to show organisms resembling those of sewage. It appeared likely that these came from the exposed foreshore, which is not far from the sewage outfall of Craigentinny Farm.

In a similar manner refuse heaps, drains, manured land and the habitations of animals, altered the character and number of organisms found in certain localities.

B.—Filtration Observations.

This method was employed on some 30 occasions, and served to demonstrate that where the gravitation method disclosed the deposit of large numbers of organisms, there the numbers per litre of air aspirated were likewise high. Suburban air showed few organisms per litre, while the central districts contained large numbers. The maximum figures, however, occurred in the Craigentinny air. The lowest numbers were found in the parks, *e.g.*, in the King's Park in the neighbourhood of Hunter's Bog, where the organisms amounted to 833 per cubic metre, or less than 1 per litre. In the same park, however, facing the Dumbiedykes houses and the wind, the filter on one day showed 1562 per cubic metre. The open spaces surrounding the town, such as Saughton Park, Blackford Hill, and Inverleith Park gave respectively 1137, 2000, and 3260 organisms per cubic metre. Open spaces in the town, such as the West and East Meadows, St. Bernard's Well, and Princes Street Gardens yielded respectively 2917, 5000, 4500, and 12,500 organisms per cubic metre. Enclosed spaces in the town, surrounded by habitations and not freely swept with air, are represented by the laboratory yard in

Forrest Road and by Surgeons' Hall. A series of 12 experiments in the former gave an average of 8096 per cubic metre, and the number found in the front of Surgeons' Hall was 15,760.

The air of Craigentinny on one occasion contained so high a number as 197,700 per cubic metre, and while this height was not reached on any other occasion, still the average in that district certainly exceeds what should be found in a suburban neighbourhood. Meteorological conditions affect the numbers of organisms denoted by this method in the same way as already shown to occur in the deposition method.

Rain has the chief influence; the lowest numbers recorded in any locality always succeed the downfall of rain. In the laboratory yard the organisms only numbered 2052 per cubic metre after rain as compared with an average of 8096. At Craigentinny, after much rain during the previous week, the numbers were reduced to 4166 per cubic metre. There is a notable contrast between this and the bacterial contents of the air of the same locality in fine weather, when the numbers never fell below 15,000 and even reached ten times that figure. Even in fine weather the air of Blackford Hill is so pure, that there are only 1 or 2 organisms present per litre.

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A CASE PRESENTING MÉNIÈRE'S SYMPTOMS ALONG WITH FACIAL PARALYSIS

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(*With* 3 *Plates*)

ALTHOUGH Ménière's (1) classical case was described as long ago as 1861, there is still considerable confusion on the subject of Ménière's disease, and the term is frequently applied to any condition in which giddiness is associated with a lesion of the ear. It would probably be better to discard altogether the use of the words "Ménière's disease," but, if the term be retained, it should be kept for cases in which Ménière's symptoms appear in an apoplectiform manner. Ménière's triad is usually described as

consisting of " noises in the ear, giddiness and vomiting," but Von Frankl-Hochwart (2), to whose valuable monograph we wish to state our great indebtedness, includes under the heading of Ménière's symptoms—" Deafness, Tinnitus, Giddiness, Nausea, Vomiting, Cerebellar Ataxia, Nystagmus, Cold Sweats, Fainting, and a Feeling of Pressure in the Head."

It canot be too emphatically stated that this " disease picture " is merely that of a lesion of the membranous labyrinth or of the eighth cranial nerve, and that it should be associated with disease of the labyrinth, just as " tremor, rapid pulse, and exophthalmos " are associated with the condition of hyperthyroidism.

In what conditions, then, do we meet with Ménière's symptoms ? The answer to this question involves an attempt at classification of diseases of the inner ear and auditory nerve, although it must be admitted that our existing knowledge is barely sufficient for this purpose ; many pathological conditions affect the aural labyrinth in which no microscopic examination of the labyrinth has yet been made.

Conditions which give rise to Ménière's Symptoms

I. *Variations in labyrinthine pressure, or excessive movements of the labyrinthine fluid*, as produced by forcible syringing, heat, cold, violent inflation of the middle ear, loud sounds, slight injuries (insufficient to cause hæmorrhage), dancing, the motion of the sea or of a railway train, etc.

II. *Electrical Stimulation.*

III. *Vascular Congestion* (*e.g.* due to amyl nitrite or otitis media, etc.) *or Anæmia* (*e.g.* quinine, general anæmia, loss of blood).

IV. *Hæmorrhage into Labyrinth*—
- *A.* Traumatic.
- *B.* Due to diminished air pressure (Caisson disease).
- *C.* In bleeding diseases, notably leukæmia, pernicious anæmia, nephritis, and arterio-sclerosis.
- *D.* In exanthemata (Politzer) (8).

V. *Acute Inflammatory Conditions*—
- *A.* Serous labyrinthitis.
- *B.* Purulent labyrinthitis.

VI. *Chronic Inflammatory Conditions*—
- *A*. Simple chronic inflammation in chronic middle-ear catarrh? Otosclerosis.
- *B*. Tubercular.
- *C*. Syphilitic: (1) congenital, (2) acquired.

VII. *Neuritis of Auditory Nerve due to*—
- *A*. Acute specific fevers.
- *B*. Tuberculosis.
- *C*. Syphilis.
- *D*. Herpetic inflammation of ganglia (Polyneuritis cerebralis Menieriformis).
- *E*. Toxic conditions—quinine, salicylates, tobacco, alcohol.

VIII. *Hæmorrhage into Auditory Nerve*—
- *A*. In bleeding diseases (includes the present case).
- *B*. In exanthemata.

IX. *Degeneration of the Auditory Nerve and Membranous Labyrinth*—
- *A*. Chronic progressive labyrinthine deafness.
- *B*. Occupational deafness.

X. *Tumours*—
- *A*. Of acoustic nerve.
- *B*. Of cerebello-pontine angle.

XI. *Neuroses*—
Pseudo-Ménière's disease (Von Frankl-Hochwart) in epilepsy, hysteria, neurasthenia, hemicrania.

Ménière's disease is the apoplectiform occurrence of Ménière's symptoms, and is usually attributed to hæmorrhage into the labyrinth. Before accepting a case as a true one of Ménière's disease, Schwartze and Grunert (3) demand that the ear shall have been normal up to the time of the occurrence of the symptoms, but Heermann (4) points out the difficulty of being sure that the ear was previously healthy, because even intelligent patients may not know that they are deaf in one ear. F. Alexander and Manasse (5) record a case in which a patient had five or six attacks of Ménière's symptoms, and, although the whisper was only heard by the right ear at one yard, the patient did not know that he had not got perfect hearing. In most cases ear disease has existed for a long

time before an attack comes on of sufficient severity to cause the patient to seek medical advice. Heermann states that out of 50 cases 27 had had previous disease of the ear. Of the 11 cases recorded by F. Alexander and Manasse, 6 showed more or less marked signs of progressive labyrinthine deafness.

Pathology.—Almost all the cases of sudden occurrence of Ménière's symptoms, in which a microscopic examination of the inner ear had been made, have been cases of leukæmia. The only non-leukæmic case reported by Von Frankl-Hochwart is that of Gruber (6)—this was a syphilitic patient who died of typhus fever: Gruber regarded the case as one of syphilitic affection, but Von Frankl-Hochwart thinks that the conditions present—vascular injection and blood-stained labyrinthine fluid (on *ma*croscopic examination)—may have been due to typhus. In a paper recently published by one of us (J. S. F.) on the subject of congenital syphilitic disease of the ear, it was noted that in several cases the deafness and other symptoms of Ménière's disease came on in a single night (29).

G. Alexander (7) claims even Ménière's original case as leukæmic: Ménière's patient was a girl, aged 16 years, who caught cold whilst driving during her menstrual period; death occurred five days after the onset of the inner-ear symptoms, and during these days the temperature was raised: at *post-mortem examination* red plastic exudation was found in the canals and, to a slight extent, in the vestibule; the cochlea was free; the brain and spinal cord were normal; no *mic*roscopic examination of the inner ear was made.

In case (13) recorded by F. Alexander and Manasse there was, however, no trace of leukæmia. The case was that of a man aged 52, who suffered from chronic progressive labyrinthine deafness with typical Ménière's symptoms a short time before death. On microscopic examination Alexander and Manasse found recent multiple hæmorrhages in the *ramus cochlearis* and *vestibularis*; the patient died of hæmorrhage into the left lateral ventricle. In addition to the hæmorrhages in the ear, atrophy of Corti's organ and of the spiral ganglion were present. There was no facial paralysis in this case.

Our case supports the view of F. Alexander and Manasse (8) that Ménière's symptoms may come on in an apoplectiform manner in non-leukæmic cases: we must note, however, that our patient suffered from chronic (senile) progressive labyrinthine deafness, and also from Eustachian obstruction on the left side.

Lucæ (9) states that in cases of chronic aural sclerosis Ménière's disease may come on suddenly without apparent cause : the patients only seek advice for the sudden attack.

Hillairet (10) was the first to point out that Ménière's symptoms might be caused by a lesion of the eighth nerve.

Von Frankl-Hochwart (pages 13 and 14) records the clinical notes of two cases of Ménière's disease in which there was paresis of one-half of the face : the second case died, but there is no mention of a microscopic examination of the ear. Similar cases are recorded by Kaufmann (11) and Hammerschlag (12). We have been able to trace the following cases of leukæmic deafness with Ménière's symptoms in which microscopical examinations of the labyrinth were made : Alt and Pineles (18) (1 case), G. Alexander (7) (15 cases), Steinbrugge (19), Mott (20), Lannois (21), Parkes-Weber and Lake (22), Schwabach (23), Politzer (8), Kock (24), and Finlayson (26). Manasse (25) examined the inner ear from thirty-one cases of nerve deafness and found two with evidence of labyrinthine hæmorrhage in addition to the usual degenerative changes. Moos (27) records the case of a syphilitic patient in whom Ménière's symptoms were engrafted on existing ear disease. Microscopical examination showed periostitis of the vestibule and small-cell infiltration of the membranous labyrinth. Haug (28) reports a tabetic case in which the acoustic nerve and ganglia were affected. Moos (13) and Steinbrugge record a case of senile dementia in which hæmorrhagic pachymeningitis was found at post-mortem : the patient had frequently suffered from giddiness. Evidences of past hæmorrhages were found in the facial canal and also between the bundles of the cochlear and vestibular nerves. Pigment was also present in the connective tissue of the membranous labyrinth. In a similar case previously recorded the same writers found, in addition to evidences of old hæmorrhages, thickening, dilatation, and, in places, obliteration of arteries of labyrinth. Both these patients had suffered from hallucinations of hearing. Ramsay Hunt (14) has described a syndrome characterised by herpes zoster oticus facialis, or occipit-collaris, with facial palsy and auditory symptoms. The same condition had previously been described by Von Frankl-Hochwart under the title of polyneuritis cerebralis Menieriformis and by numerous other writers. In one case Ramsay Hunt (15) demonstrated sclerosis of the nerve of Wrisberg on microscopical examination.

Present Case

A. M'R., male, piermaster, aged 65; patient suffered from measles and typhoid as a child, and for the last few years from rheumatism and from left inguinal hernia; no history of syphilis; his left eye was removed thirty years ago; patient stated that he had never suffered from deafness up to the time of the last illness; father and mother died of old age, one brother of cancer, and one sister of "water in the head."

There has been some difficulty in getting an accurate history of the case, but after extended inquiry we have arrived at the following account, which may be accepted as correct:—

On 1st December 1907 he had a long day of exposure to cold on the pier, but went to bed at night feeling well. The following morning (2/12/07) he woke up suffering from extreme giddiness and from deafness and noises in the left ear; the left side of his face was paralysed. No history of vomiting was obtained. Patient stated that giddiness was the worst symptom of all; he also complained of pain in the head and limbs, and did not leave his bed for a week. While in bed he sometimes felt as if surrounding objects were moving round him.

When he did get up he had to hold on to various articles of furniture in order to get about the house; he states that he tended to fall to the right side—the side opposite to the ear lesion.

Towards the end of December 1907 the deafness got worse.

Patient's doctor was not consulted till 6th January 1908, and patient was not examined at the Royal Infirmary till 11th February, when he was seen by Dr Logan Turner at the Ear and Throat Department, at the request of one of us (A. B.). Dr Turner's notes are as follows:—Tympanic membranes are both slightly indrawn, but are otherwise normal. On the right side the watch is heard at two inches, but on the left it is not heard even on contact with the auricle. Forced whisper heard by right ear at two feet and by left ear at one foot. All tuning-forks heard by air conduction by left ear. Rinne's test positive on both sides. On inflation with the catheter air did not enter the left tympanic cavity—right side not tested. Patient can stand with his feet together and eyes shut without swaying, but he is unable to stand on either foot alone without tending to fall. Patient suffers from left facial paralysis, but can taste salt on the anterior part of the tongue on the left side. *Herpes auricularis* was not present: this is interesting, and must be

taken along with the retention of the sense of taste on the same side of the tongue. As will be seen from the account of the microscopical examination of the inner ear, the hæmorrhage did not extend beyond the internal meatus, and did not involve the facial nerve in the aqueduct of Fallopius nor the geniculate ganglion.

The patient was admitted to Dr Bruce's ward on February 18, 1908; on admission he stated that he was giddy, even when lying in bed, but that the giddiness was worse in the open air, especially when passing vehicles.

Examination.—Patient somewhat emaciated; complete left facial paralysis; left eyebrow and left corner of mouth are depressed; no response to the faradic current on the left side of the face; the affected muscles contract slowly to galvanism, but there is no alteration of the polar reaction; superficial and deep reflexes present and active; voluntary movements are weak, and hand grip is weaker on left than on right side; *arterial walls thickened*; slight rough presystolic murmur in mitral area; aortic second sound accentuated and reduplicated. *No evidence of leukæmia.*

Urine, specific gravity 1014; otherwise normal.
Lungs, both reveal extensive chronic tuberculosis.
Joints of hands swollen and painful.

The diagnosis made by one of us (A. B.) was that of neuritis of facial and auditory nerves in the internal auditory meatus; hæmorrhage in this situation was also mentioned as a probable cause of the symptoms. In the *Review of Neurology*, Drs Bruce and Pirie write: "The absence of evidence of middle-ear disease and the retention of the sense of taste, and the associated deafness, giddiness, and facial paralysis indicated that the lesion was probably situated between the side of the pons and the bottom of the internal auditory meatus."

Treatment.—Potassium iodide 10 grs. t.i.d.
Galvanic electricity to left side of face.

Progress.—4/4/08. Giddiness and facial paralysis *in statu quo.*
9/4/08. Irritable dermatitis on legs.
10/4/08. Pot. iod. stopped: aspirine, 10 grs. t.i.d.
12/4/08. Patient still feels very giddy if he sits up in bed.

17/4/08. Patient allowed to get up: does not tend to fall to right as much as formerly. Constant current passed from mastoid to mastoid daily.

24/4/08. Patient sent to Convalescent House; giddiness still present; he still suffers from rheumatism.

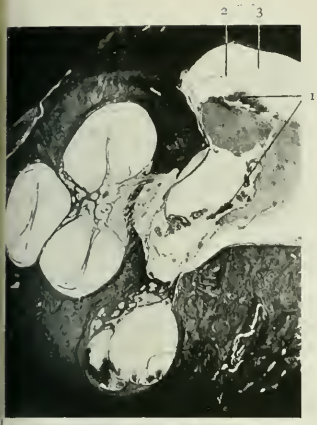
2/5/08. Patient died suddenly at Convalescent House from syncope this morning.

Post-mortem examination held forty-eight hours after death.

Summary.—Atrophy of second, third, fourth, and sixth cranial nerves on left side. Fatty dilated heart. Chronic venous congestion of organs. Extensive chronic pulmonary tuberculosis. Subacute interstitial nephritis. Brain shows definite milky thickening of arachnoid over inferior surface of cerebellum and circle of Willis; this condition extends 1¼ inches in front of optic chiasma and also to near the tip of the temporal lobe.

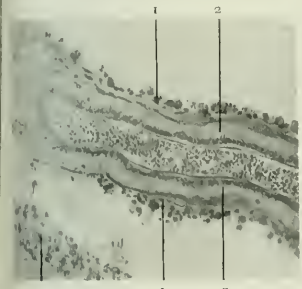
Drs Bruce and Pirie (16) found a well-marked Nissl's *réaction à distance* limited to the facial nucleus of the same side only and a small nucleus behind it; the hypoglossal nucleus and the nucleus of the third nerve on the same and opposite sides were intact—thus showing conclusively that the facial nucleus is the only nucleus of the facial nerve.

Examination of Left Inner Ear and Auditory and Facial Nerves.—The left temporal bone was removed at the post-mortem on 4th May 1908, *i.e.* forty-eight hours after death; it was at once placed in 10 per cent. formalin, where it remained till 25th March 1909. The inner ear spaces were then opened (J. S. F.), and the petrous bone was prepared for microscopic examination according to Ruttin's (17) method. The inner ear was decalcified in 10 per cent. nitric acid and 10 per cent. formol āā for two months—the fluid being changed daily at first. The specimen was then washed for a week in running water, and placed in 70 per cent. spirit for a day or two; next transferred to 90 per cent. spirit for three days (changed) daily; then absolute alcohol for forty-eight hours (changed once); then absolute alcohol and ether for forty-eght hours (changed once); next thin celloidin for one month, and finally thick celloidin for one month. After the thick celloidin had hardened, the superfluous parts were removed and the block cut in the vertical transverse direction, *i.e.* at right angles to the long axis of the petrous pyramid. In all about 600 serial sections were cut—20



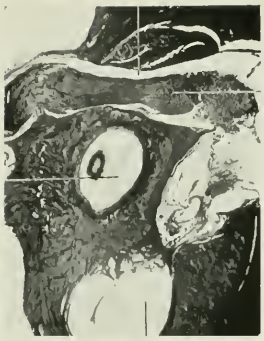
Vertical Section of Cochlea and Internal Auditory Meatus, showing Hæmorrhage.

1. Hæmorrhage beneath epineurium. 2. Epineurium. 3. Space between epineurium and ura.



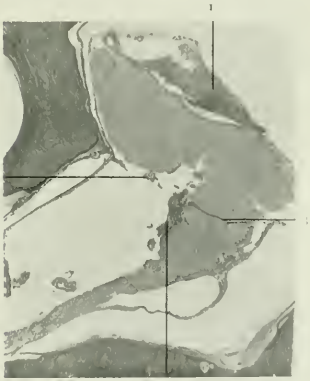
Longitudinal Section of Artery in Internal Auditory Meatus.

1. Hæmorrhage. 2. Thickened middle coat of artery.



Facial Nerve (in Fallopian Canal) *passing above Cochlea* (note absence of hæmorrhage).

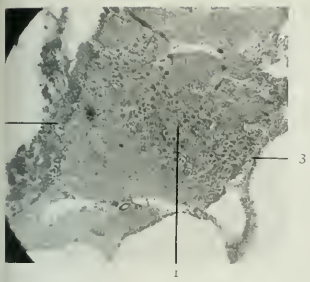
1. Facial nerve. 2. Space between epineurium and wall of canal free from hæmorrhage. 3. Middle coil of cochlea. 4. Basal coil.



Seventh and Eighth Nerves in Internal Auditory Meatus.

1. Hæmorrhage beneath epineurium of seventh nerve. 2. Thickened artery. 3. Hæmorrhage around vestibular ganglion. 4. Vein.

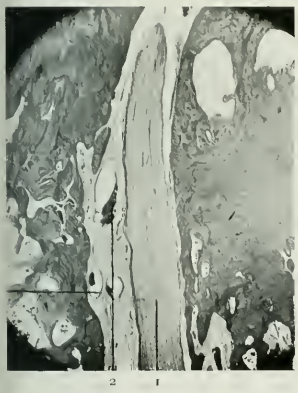
PLATE 4.—TO ILLUSTRATE ARTICLE BY DR BRUCE AND MR FRASER.



1. Vestibular ganglion. 2. Hæmorrhage. 3. Epineurium.

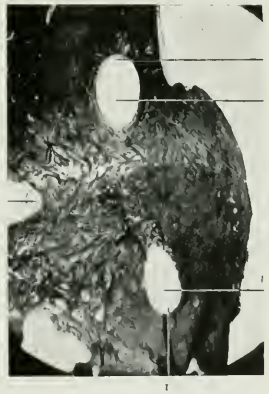
Vertical Section of Vestibule.

1. Membrane of round window. 2. Basal membrane (note absence of Corti's organ). 3. Perilymphatic space of vestibule. 4. Neuro-epithelium of anterior wall of utricle. 5. Branch of vestibular nerve to utricle. 6. Vessel. 7. Facial nerve. 8. Footplate of stapes. 9. Promontory.



Descending portion of Facial Nerve in Canal of Fallopius.

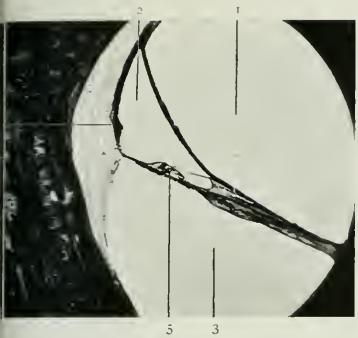
1. Facial nerve. 2. Vessels.



Semicircular Canals, showing absence of Hæmorrhage.

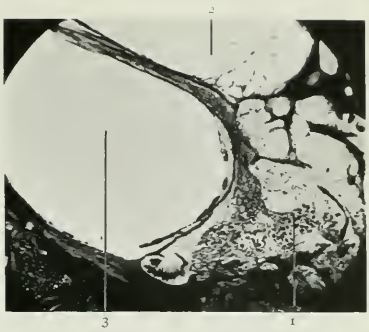
1. Membranous canals. 2. Perilymphatic space.

PLATE 5.—TO ILLUSTRATE ARTICLE BY DR BRUCE AND MR FRASER.



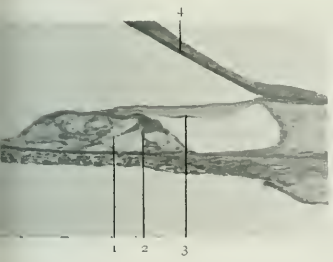
Three Scalæ of Cochlea from Middle Coil.

Scala vestibuli. 2. Scala media (cochlear canal). cala tympani. 4. Epithelial cells covering stria laris. 5. Basilar membrane.



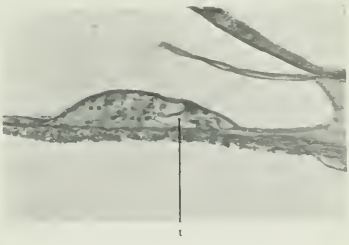
Spiral Ganglion of Middle Coil of Cochlea.

1. Cells of ganglion. 2. Scala vestibuli. 3. Scala tympani.



Corti's Organ from Middle Coil of Cochlea.

Outer pillar cell. 2. Inner pillar cell. 3. Membrana tectoria. 4. Reissner's membrane.



Corti's Organ from Middle Coil of Cochlea.

1. Nerve fibre crossing tunnel of Corti.

PLATE 6.—TO ILLUSTRATE ARTICLE BY DR BRUCE AND MR FRASER.

μ in thickness—and about 150 were stained with hæmalum and eosin or by Van Gieson's method. Owing to the want of fixation in Muller's fluid and the prolonged soaking in formol it was found impossible to get good results with the Pal-Weigert method. In spite of the fact that the temporal bone was only obtained forty-eight hours after death, it will be seen that the structures of the membranous labyrinth were well preserved. In forming an opinion as to the size of the hæmorrhage in the internal meatus and the condition of the auditory, vestibular, and facial nerves, it is necessary to remember the prolonged soaking of the specimen in formol solution for the period of nearly one year.

Briefly summed up, the result of the microscopic examination of the inner ear and auditory and facial nerves was as follows :— The inner ear was almost normal ; there was no hæmorrhage in the endo- or peri-lymphatic spaces of the cochlea, vestibuli or canals ; the membranous labyrinth was also almost normal, as was the spiral ganglion ; the neuro-epithelium of the utricle, saccule, and of the cristæ ampullarum was well preserved. In the lower turn of the cochlea Corti's organ was absent or degenerated. The mucous membrane of the middle ear was normal.

As will be seen from the result of Dr Logan Turner's examination of the patient, the case was probably one of chronic progressive (labyrinthine) deafness.

In the internal meatus there was a considerable amount of hæmorrhage between the epineurium and the seventh and eighth nerves : this was most marked above the seventh nerve. There was no hæmorrhage between the dura mater and epineurium. The vessels in the internal auditory meatus were markedly thickened and the muscular coat had undergone a hyaline change. The hæmorrhage did not extend into the Fallopian canal, and did not therefore affect the geniculate ganglion : the modiolus of the cochlea and the small canals for the vestibular nerves were also free from hæmorrhage. Reference to the reproductions of the photomicrographs will demonstrate the pathological condition present better than a more detailed description.

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POST-OPERATIVE PERITONITIS.

THE PROPHYLACTIC USE OF BACILLUS COLI VACCINE AND NUCLEIC ACID.*

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In spite of the great advances which have of recent years been made in the field of intestinal surgery, we have to acknowledge to-day a death rate of 20 per cent. in cases of intestinal resection. The major part of this mortality is due to peritonitis, the infection occurring either at the time of operation, or a few days later, from a leak in the suture line.

Apart from operations of emergency, post-operative peritonitis is to be feared chiefly after resections of large intestine, and after operations for the cure of fæcal fistulæ, where the asepsis of the field of operation is uncertain. Although the success of such operations must always depend on the skill and judgment of the operator, and although the patient's fate is usually decided at the operation, we are nevertheless justified in endeavouring to find some means of preparing the patient before operation with a view to putting him in a better position to withstand infection of his peritoneal cavity, should it occur at, or subsequent to, the operation. This paper deals with the evidence in favour of the use of certain measures designed for that purpose. The subject of post-operative peritonitis is a

* The expenses of the experimental part of this research were defrayed by a grant from the Carnegie Trust.

gloomy one, for we know that this complication of intestinal surgery is almost uniformly fatal, and that operative interference for its relief is notoriously unsatisfactory. As a rule the patient's only chance lies in a rapid peritoneal reaction round the primary site of infection, with the formation of adhesions walling it off from the rest of the peritoneal cavity. Now the occurrence or the absence of a prompt peritoneal reaction, with the formation of plastic adhesions round the site of a sudden infection, depends partly on the number and the virulence of the bacteria which have escaped into the peritoneal cavity and partly on the resistive power of the patient for these bacteria. From animal experiments we know in how prompt and efficient a manner the peritoneum reacts when it has to deal with a type of infection to which the animal has been previously immunised, or to which it is naturally not very susceptible, and on the other hand how the reactive powers of the peritoneum appear paralysed when called on suddenly to deal with a virulent infection to which the animal has a low resistive power. In the latter case the bacteria appear to multiply rapidly and to get a firm footing before the peritoneum has time to throw out protecting lymph to form a barrier or phagocytic cells to combat the bacteria. On preparedness for prompt reaction, therefore, depends the patient's fate in cases of post-operative peritonitis. The methods of preparing the patient to withstand infection which are here dealt with, are based on certain pathological data which require consideration.

Bacteriology of Post-Operative Peritonitis.

It is found in cases of peritonitis following intestinal operations that the Bacillus Coli Communis is the predominating organism in the peritoneal exudate. Many observers have regarded this organism as being of secondary importance and have attributed the fatal issue so frequent in these cases, to the presence of the streptococcus or to some anærobe. There is now, however, no room for doubt that these observers greatly under-estimated the virulence of the Bac. Coli when it is multiplying rapidly in the peritoneal cavity. It has of late been frequently demonstrated that in a generalised peritonitis, Bac.

Coli may be the only organism discoverable both by film preparations and cultures from the exudate, and moreover that in such cases a fatal issue is the rule. The investigations of Dudgeon and Sargent have given strong support to this view. We have also every reason to believe that, should a quantity of a certain strain of Bac. Coli of comparatively low virulence escape into and gain a hold in the peritoneal cavity of a patient with low resistive power, the bacillus, and consequently its toxins, will increase in virulence as it multiplies and will eventually kill the host. In any case, even though this bacillus be not the most virulent of the germs which have escaped into the peritoneal cavity, its presence there appears to favour the growth of the other bacteria. It is, therefore, the germ against which the patient most needs protection.

The Importance of a Prompt and Vigorous Reaction in the Peritoneal Cavity, in Cases of Peritonitis.

Too strong emphasis cannot be laid on the value of a rapid and profuse emigration of phagocytes into the peritoneal cavity in infections of that cavity, and we now recognise that the cases of peritonitis with the worst prognosis are not those in which the peritoneal exudate is thick and purulent, but those where it is scanty, clear and serous but laden with germs. The following experiment (although intended for another purpose) brought out these points so clearly that it is here recorded in detail.

Experiment I.

6. i. 09. To each of three full grown rabbits an intraperitoneal injection of a bacterial emulsion consisting of 1500 million staphylococci and 50 million pneumococci was given (this was $1\frac{1}{2}$ times the lethal dose of this combination of bacteria). On January 7th, 24 hours after injection, peritoneal fluid was withdrawn from each rabbit by means of a capillary glass pipette and films were made. It was now found that one of the rabbits (rabbit 1) had been suffering from a subcutaneous abscess on the left gluteal region which was, however, practically healed. No staphylococci or pneumococci were found in what little discharge there was from the abscess. This rabbit (1) appeared

quite well and ate heartily on the morning after the peritoneal injection; the other two rabbits looked dull and did not eat. The peritoneal fluid from rabbit 1 was turbid and purulent, from rabbits 2 and 3 it was clear and serous, and microscopic examination revealed the fact that whereas in the fluid from rabbit 1 polymorph leucocytes showing active phagocytosis abounded, in the fluid from rabbit 2 there were very few polymorphs, and in that from rabbit 3 only a limited number. On January 8 (48 hours after injection) the difference between the animals was still more striking. Rabbit 1 now appeared quite well, whilst rabbit 2 was very dull and abdominal looking, and rabbit 3 though better than 2 was not eating. Peritoneal fluid was again withdrawn from the three rabbits. In rabbit 1 it was thick and purulent, from rabbits 2 and 3 it was serous and showed only very slight turbidity. Microscopic examination showed that the fluid from 1 was crowded with polymorphs, most of which contained cocci, and very few cocci remained unphagocyted. The film from rabbit 2 showed very few polymorphs, but many cocci free in the fluid, whilst in the fluid from rabbit 3 there were more polymorphs but also many free cocci. Rabbit 2 died 58 hours after the start of the peritoneal infection, and p.m. showed a purulent plastic peritonitis. Its heart blood was sterile. Examination of the blood of rabbits 1 and 3 now showed that the former had a leucocytosis of 19,000, the latter no leucocytosis (7000) and differential counts showed that the polymorphs in rabbit 1 averaged 75 per cent., in rabbit 3 only 60 per cent. Rabbit 3 died $3\frac{1}{2}$ days after the injection and post mortem, a condition of pyæmia was found with abscesses in lungs and liver. Rabbit 1 lived for $8\frac{1}{2}$ days after infection; post mortem a purulent plastic peritonitis was found: the heart blood was sterile.

This experiment brings out very clearly several important points. The first is that the presence of a leucocytosis in the blood favours a good leucocyte emigration into the peritoneal cavity. (That is as one would expect.) The second is that a good cellular emigration into the peritoneal effusion is one of the chief factors making for success in the combat with the invading germs. Thirdly, that from the naked eye appearance

of the peritoneal fluid, one cannot judge as to the extent or virulence of the infection. It was noteworthy that whilst in rabbit 1 turbid purulent fluid was withdrawn on the second day, in rabbits 2 and 3 the fluid was almost clear.

The purulent character of the fluid was evidence, not of the severity of the infection, but of the intensity and vigour of the rabbit's resistive powers.

Incidentally one would draw attention to the practical bearing of this on the surgical treatment of peritonitis. On opening the abdomen of a patient suffering from peritonitis free sero-purulent fluid is found. (The source of the infection is immaterial.) One cannot tell from naked eye examination, whether that fluid is germ free and protective, or whether it is germ-laden and a menace to the patient. The only satisfactory test is an immediate microscopic examination. This can be made in the operating theatre in 3 minutes. If the film shows numerous undegenerated cells and no free micro-organisms, then the fluid is protective and should be left alone. If on the other hand, the cells are beginning to degenerate, and free cocci or bacilli are seen, then the fluid is a menace to the patient and douching out of the abdomen is indicated. To put it briefly :—Before washing out an abdomen, one should know what one is washing out, and before refraining from washing out an abdomen, one should know accurately what one is leaving behind in it. The knowledge gained from an immediate microscopic examination of the fluid is also invaluable in aiding an accurate prognosis in such cases.

The Value of Preliminary Injections of Vaccines in Preventing Fatal Peritonitis.

If we grant that the Bac. Coli plays an all important rôle in peritonitis following intestinal operations, the question now arises: Could we by giving injections of a vaccine made from a virulent strain or from several strains of B. Coli, to a patient before an intestinal operation, raise his resistive power to post-operative peritonitis? Several experiments were carried out on animals with a view to testing this question, and the results of these observations are given below.

The experiments were carried out on rabbits and guinea pigs, and the following method was employed:—The lethal intraperitoneal dose of a certain strain of bacteria (staphylococcus aureus for rabbits, B. Coli for guinea pigs) was first ascertained. In one series of animals preliminary subcutaneous injections of a vaccine, made from cultures of the bacteria to be used later in inducing peritonitis, were given. Slightly more than the lethal dose of the bacterium was then injected into the peritoneal cavity of each of the vaccinated animals and an equal dose from the same culture was likewise injected into the unvaccinated control animals. The experiments extended over several weeks and as the virulence of the bacteria varied considerably from day to day a fresh control animal was used for each experiment. In some of the experiments only one preliminary vaccine injection was given, in others several injections were given, at intervals of 6 or 7 days, before inducing peritonitis. In most cases the vaccine used was made from the same virulent strain of bacteria as was used for intraperitoneal injection, in a few cases, however, a vaccine made from an old stock, non-virulent, laboratory culture of the same organism was used. Twelve such experiments were carried out. In three of these experiments the animals which had received the preliminary vaccine injection survived, whilst the controls died of peritonitis. In two experiments the vaccinated animals made prompt recoveries, whilst the controls were ill for two or three days but then recovered. In two experiments both the vaccinated animals and the controls made prompt recoveries. In one experiment both the vaccinated animal and the control died at practically the same length of time after the induction of peritonitis. In two experiments the controls lived, whilst the vaccinated animals died. In one of the latter, however, two enormous doses of vaccine had been given shortly before the peritonitis was induced, and whilst a marked negative phase, as shown by opsonic examination, was still present. In the other case the vaccinated animal died of acute enteritis which may or may not have been caused by the peritonitis. In most cases daily opsonic examinations were made, but the only important point brought out by these was that the state of the opsonic index for the infecting bacteria was

of no prognostic value. Thus some of the animals which died had a very high index, whilst in others which recovered, the index was low. This but confirmed what one had found in the human subject in acute abdominal infections. From these experiments one concluded that the preliminary injection of a suitable vaccine did give some protection against death from peritonitis, provided the vaccine was given in reasonable dosage, and provided a sufficiently long interval was allowed to elapse between the injection of the vaccine and the onset of peritonitis. One, therefore, felt justified in using this method in the human subject in cases where an intestinal resection was anticipated. Accordingly this method was employed in eight cases of resection of large intestine. A vaccine was made from a virulent strain of Bac. Coli obtained from a case of acute appendicitis. One or more injections, each of 150 million dead bacteria, were given some days before operation. It was found advisable to allow at least two days to elapse between the vaccine injection and the operation, to ensure the negative phase being over.

No conclusions as to the value of preliminary vaccine injections could possibly be drawn, however, from such a small number of cases, and all one can say is, that such injections produced no untoward effects.

Artificial Stimulation of Leucocytosis before Abdominal Operations.

Under this heading two forms of leucocytosis demand consideration; firstly a peritoneal leucocytosis, and secondly a leucocytosis in the general blood stream. The wonderful power of reaction to any form of irritation, possessed by the peritoneum has long been a matter of common knowledge. Issaeff showed that the cellular reaction produced by the intraperitoneal injection of such harmless agents as sterile saline and broth, or still more so, by nuclein and its allies, would protect an animal *twenty-four hours later* against the intraperitoneal injection of ten times the lethal dose of the cholera spirillum.

Issaeff's results have been confirmed and amplified by very numerous observers, especially Borchard, and by Diez and Compara, and we know that the intraperitoneal injection of

practically any non-lethal substance will produce a peritoneal leucocytosis and will protect the animal 24 hours later against the injection of one or more lethal doses of bacteria. This striking discovery is surely a very strong argument in favour of performing serious abdominal operations in two stages if posible. For example, if the abdomen be explored and a large adherent but still removable carcinoma of the cæcum or ascending colon be found, in the light of Issaeff's work it would be advisable to make a lateral anastomosis between the ileum and transverse colon, to pour saline into the abdomen, and several days later to excise the diseased portion of bowel. The peritoneum would thus be prepared for any infection which might take place during the more difficult part of the operation. Though well aware of the clinical disadvantages of such a method, one feels sure that the risk of death from peritonitis which in such cases is by no means small, would be considerably lessened.

Mickulicz, in one or two cases, gave intraperitoneal injections of nucleic acid to patients the day before performing an abdominal operation on them. He very quickly came to the conclusion, however, that the risk and discomfort attending this method, disqualified it for general use.

Mijake, working in Mickulicz' laboratory, found that in guinea pigs the *subcutaneous* injection of nuclein produced a leucocytosis in their blood, and protected them to some extent (2-3 times the lethal dose) against peritonitis induced 9 hours later.

This method of subcutaneous injection of 2-6 cc. of 5 per cent. nucleic acid, Mickulicz extensively practised as a preliminary measure to abdominal operations, with, he thought, improved results. His untimely death, however, cut short his observations.

The subcutaneous injection of nucleic acid in the human subject and in animals is followed fairly constantly by definite changes in the blood. For the first few hours (1 hour—6 hours) there is a well marked leucopænia, the leucocyte count frequently falling from 8000 down to 4000. The leucocytes in the blood then rapidly increase in number and may rise in the human subject, 12 hours after the injection, to 25,000. In rabbits and

guinea pigs, they seldom rise above 15,000, and frequently do not reach that figure. The increase is found to be almost entirely in the polymorph leucocytes.

The Effect of the Subcutaneous Injection of Nucleic Acid on the Peritoneum.

It is certainly of interest and importance to know whether, along with the leucocytosis in the blood, any increase in the number of cells in the peritoneal cavity takes place after the subcutaneous administration of nucleic acid. To ascertain this the following experiment was carried out.

Experiment II.

Two rabbits were each given 2 cc. of 5 per cent. nucleic acid simultaneously; a third rabbit was used as a control. Peritoneal fluid was drawn off hourly by means of a capillary pipette from one of the "nuclein rabbits" (1), and from the control (3). The other "nuclein rabbit" (2) was left alone until a leucocytosis in its blood was established.

In rabbits 1 and 3 after the first three hours, a polymorph leucocytosis in the peritoneal cavity began to be evident, and this, during the next 3-4 hours became more and more marked (due no doubt, to the repeated irritation caused by the punctures). It was, however, much more marked in rabbit 1, which had received the nuclein, than in rabbit 3 which had not.

After seven hours (a leucocytosis in the blood being already present) peritoneal fluid was, for the first time, drawn off from rabbit 2, but not a single polymorph leucocyte was found in the films from the fluid obtained. This showed that nuclein does not produce a leucocytosis in the peritoneal cavity when injected subcutaneously, unless the factor of some local irritation of the peritoneum be introduced, but, given this latter factor, an emigration of polymorphs into the peritoneal cavity is favoured and hastened by the presence of a leucocytosis in the blood. This observation has been repeatedly confirmed.

In rabbits and guinea pigs one has found that though the preliminary subcutaneous administration of nucleic acid does

give some protection against peritonitis, this protection is not very striking nor is it quite constant. This will be seen from the experimental results detailed later.

During the years 1905 and 1906, Mr. Caird employed this method of preliminary injection of nuclein in most of his abdominal cases in the Edinburgh Royal Infirmary. He has kindly permitted me to look through the notes on these cases.

In all of them 8 cc. of 5 per cent. nucleinic acid (Park Davis) was given at approximately twelve hours before operation. The injections were often followed by considerable discomfort; it has since been found, however, that by employing a 2 per cent. isotonic solution of this drug all discomfort may be avoided. Leucocyte counts were made in all cases before the injection of the drug and again just before operation. In 60 cases so treated, some degree of leucocytosis was invariably produced by the nuclein injection. The leucocytosis varied from 10,000 up to (in one case) 26,000, the average for all the cases being 15,500. Owing to the varied nature of the cases so treated it was of necessity, very difficult to judge exactly how much benefit they derived from the nuclein injections. One could only say that having a leucocytosis in their blood at the time of operation, in the light of our experimental evidence, they were presumably in a better condition to withstand peritoneal infection, than would otherwise have been the case.

The Combination of Vaccine and Nuclein Injections as a Protective Measure against Peritonitis.

Several experiments were carried out to test whether the administration of both vaccine and nuclein before the onset of a peritonitis would give greater protection against a fatal issue than was given by either of these agents employed alone. As will be seen from the records of experiments given below, this combination proved to be greatly superior to either vaccine or nuclein alone, so much so that one is encouraged to hope that it may prove to be of considerable value in human surgery.

The first of these experiments is here recorded in detail, the others are given in tabular form.

Experiment I.

Four rabbits were used, and before inducing peritonitis the following treatment was given :—

Rabbit 1 was given subcutaneously vaccine and nucleic acid.

Rabbit 2 was given subcutaneously vaccine alone.

Rabbit 3 was given subcutaneously nucleic acid alone.

Rabbit 4 was on control.

Rabbits 1, 2 and 3 were of equal size and weight. Rabbit 4 was larger, weighing 100 grms. more; it was therefore made the control.

Dec. 4th, 08, 12 noon. Rabbits 1 and 2 each given a subcutaneous injection of staphylococcus aureus vaccine (660 million cocci).

Dec. 6th, 5 p.m. Rabbits 1 and 3 each given a subcutaneous injection of 2 c.cm. 5 per cent. nucleic acid.

Dec. 7th, 08, 2.30 p.m. All four rabbits given an intraperitoneal injection of 14,000 living staphylococci.

Rabbit 1 never showed any signs of illness and made a complete recovery.

Rabbit 2 began to look dull two hours after the injection and died within seven hours. Post mortem :—staphylococci grown on culture from peritoneal fluid and from heart blood.

Rabbit 3 remained well for $3\frac{1}{2}$ hours, then began to be dull and died 10 hours after injection. Post mortem :—same finding as in rabbit 2.

Rabbit 4 (control) was fairly well for 10 hours, then became very dull and died 16 hours after injection. Peritoneal fluid was drawn off every hour from all four rabbits from the time of injection until the death of rabbits 2, 3 and 4, and films from this were examined. It was seen that whereas in rabbit 1 the cells in the peritoneal fluid were plentiful and soon obtained the upper hand of the cocci, in rabbits 2, 3 and 4 the cells were much less numerous, and after a stage of uncertainty, lasting for about one hour, during which the cells and the bacteria appeared to be equally numerous, the latter multiplied rapidly and the cells degenerated until just before death the peritoneal fluid was swarming with cocci and but few cells were to be seen.

This experiment again showed very clearly how in severe

peritoneal affections the fate of the animal depends very largely on the vigour of the *initial* reaction, and that apparently the preliminary treatment with vaccine and nuclein favoured a prompt and successful reaction.

Experiment II.

In this experiment in order to make the peritonitis more fully resemble post-operative peritonitis of the human subject, an abdominal operation was performed on each rabbit (under ether anæsthesia), its appendix being removed, just before the intra-peritoneal injection of cocci was given.

EXPERIMENT II.

Animal.	Preliminary Injection of	Intraperitoneal Injection of	Result.
Rabbit I.	Vacc. and Nuclein.	3,000 mill. Staph. Aur.	Recovery.
Rabbit II.	Vaccine only.	„	Death 20 hrs. after injec.
Rabbit III.... ...	Nuclein.	„	Death 8 days after injec.
Rabbit IV.... ...	Control.	„	Death 18 hrs. after.

In this experiment (II) the animal which had preliminary treatment with vaccine and nuclein never showed any ill effects from the operation or subsequent peritonitis.

In *Experiment III.* no operation was performed before the induction of peritonitis.

EXPERIMENT III.

Animal	Preliminary Injection of	Intraperitoneal Injection of	Result.
Rabbit I.	Vacc. and Nuclein.	4,500 mill.	Prompt recovery.
Rabbit II.	Vaccine only.	„	Death 5 days later.
Rabbit III.... ...	Nuclein only.	„	Death 10 hrs. after injec.
Rabbit IV.... ...	Control.	„	Ill but recovered.

The following three experiments were carried out on guinea pigs, and a moderately virulent strain of Bac. Coli Communis was the bacterium used. An additional factor was introduced into these experiments, in that the relative values of a vaccine made from a virulent strain of B. Coli and one made from an old stock laboratory culture were tested. Six guinea pigs were used in each experiment so that the relative values of the two vaccines when used alone and when in conjunction with nucleic acid might be observed. The vaccine injections were in each experiment given two days, and the nucleic acid injections 14 hours, before peritonitis was induced. Both injections were of course given subcutaneously.

EXPERIMENT IV.

Animal.	Preliminary Treatment	Intraperitoneal Injection of	Result.
G.P. I.	..."Virulent" Vacc. +Nuclein.	600 million virulent B. Coli.	Prompt recovery.
G.P. II. ...	..."Virulent" Vacc. alone.	„ „	Dull for a few hours but recovered.
G.P. III. ...	..."Stock" Vaccine +Nuclein.	„	Ill for 24 hrs. but recov'd.
G.P. IV. ...	..."Stock" Vaccine alone.	„	Very dull for 2 days but recovered.
G.P. V. ...	..."Neuclein"alone	„	Died 20 hrs. after injec.
G.P. VI. ...	... Control.	„	Very dull for 3 days, eventually recov.

In this experiment (IV) the animal which received the injections of virulent vaccine and nuclein never showed the slightest indisposition and twelve hours after the infection could at once be picked out from all the others.

EXPERIMENT V.

Animal.	Preliminary Treatment.	Intraperitoneal Injection of	Result.
G.P. I.... ...	..."Virulent" Vacc. + Nuclein.	1,100 million virulent B. Coli.	Recovered and was well 8 days after infection.
G.P. II. ...	..."Virulent" Vacc. alone.	„	Died 15 hours after injec.
G.P. III. ...	..."Stock" Vaccine + Nuclein.	„	Died 19 hours after injec.
G.P. IV. ...	..."Stock" Vaccine alone.	„	Died 11 hours after injec.
G.P. V. ...	... Nuclein alone.	„	Died 13 hours after injec.
G.P. VI. ...	... Control.	„	Died 10 hours after injec.

Here again, the animal which received the virulent vaccine and nuclein differed markedly from all the others.

EXPERIMENT VI.

Animal.	Preliminary Treatment.	Intraperitoneal Injection of	Result.
G.P. I.	..."Virulent" Vacc. + Nuclein.	1,100 million „	Slightly ill for 24 hours, recovery.
G.P. II.... ...	..."Virulent" Vacc. alone.	„	Ill for 30 hrs., recovery.
G.P. III. ...	..."Stock" Vaccine + Nuclein.	„	Slightly ill for 30 hours, recovery.
G.P. IV. ...	..."Stock" Vaccine	„	Very ill for 3 days, recovery.
G.P. V.... ...	... Nuclein alone.	„	Very dull for 3 days, recovery.
G.P. VI. ...	... Control.	„	Died 30 hours after injec.

Recognising that the injection of a virulent vaccine and nuclein did give considerable protection against death from peritonitis, one next wished to ascertain what the extent and limits of that protection were. The following experiment (VII) was therefore carried out with that purpose.

Experiment VII.

The minimal lethal dose of a virulent B. Coli for a guinea pig of a certain weight was ascertained by giving graduated doses to a series of those animals.

The minimal lethal dose of the Bac. Coli used in this experiment (VII) was 700 million bacilli for a guinea pig weighing 500 grms.

Multiples of this dose of B. Coli were injected into eight guinea pigs, four of which had received preliminary treatment with virulent vaccine and nuclein, the other four acting as controls.

EXPERIMENT VII.

	Animal.	Weight.	Preliminary Treatment.	Intraperitoneal Injection of	Result.
I.	G.P. I. ...	410 grm.	"Vir." Vacc. + Nuclein.	1,000 million virulent B. Coli.	Prompt recovery.
	G.P. (Control)	450 grm.	Nil.	„	Death 19 hrs. after injec.
II.	G.P. II....	480 grm.	"Vir." Vacc. + Nuclein.	1,500 million virulent B. Coli.	Very ill for 2 days, recovery.
	G.P. (Control)	500 grm.	Nil.	„	Death 17½ hours after injection.
III.	G.P. III. .	440 grm.	"Vir." Vacc. + Nuclein.	2,000 million virulent B. Coli.	Ill for 24 hrs. but recov'd.
	G.P. (Control)	490 grm.	Nil.	„	Death 16 hrs. after injec.
IV.	G.P. IV. .	600 grm.	"Vir." Vacc. + Nuclein.	2,500 million virulent B. Coli.	Ill for 3 days but recov'd.
	G.P. (Control)	680 grm.	Nil.	„	Death 25 hrs. after injec.

It should be noted that each of the control guinea pigs weighed more than the corresponding animal which had received preliminary treatment.

From Experiment VII. it is seen that preliminary treatment with vaccine and nuclein gave protection against rather more than three times the lethal dose of Bac. Coli.

The results obtained with this combination of vaccine and nuclein were sufficiently striking to warrant its employment in the human subject before intestinal operations.

Vaccine and Nuclein Injections in the Human Subject.

Through the kindness of Professor Caird I have had the opportunity to employ this method in the human subject before operations for resection of large intestine in 16 cases, and, so far as one could judge, with benefit to the patients. The cases comprised: resection of the sigmoid for carcinoma, 7 cases; excision of the rectum by the abdomino-perineal route, 5 cases; resection of intestine for cure of fæcal fistula, 4 cases.

Of these 16 cases, 14 recovered, and 2 died within ten days of operation. Death was due in one case to sudden heart failure, and in the other to chronic intestinal toxæmia. Post mortem examination in both the fatal cases confirmed the absence of any peritonitis.

It was also practised before operation for gall-stones and cholecystitis, 3 cases; and perineal excision of the rectum, 3 cases. The method is particularly applicable in cases of chronic obstruction due to a malignant stricture of the great gut, where, owing to the fæcal accumulation above the growth, a preliminary colostomy must be performed to allow of a thorough clearing out of the bowel above the growth, before the radical operation for its removal is undertaken.

Such cases are of very frequent occurrence, and the danger of infecting the peritoneal cavity at the second operation, which is conducted alongside a fæcal fistula, is a very real one. The time which intervenes between the performance of the colostomy operation and of the second operation for removal of the neoplasm may be well employed by raising the patient's resistance to a B. Coli infection by the administration of one or more doses of coli vaccine and of nucleic acid. The following case illustrates the use of this method.

Acute Intestinal Obstruction from an Annular Carcinoma of the Pelvic Colon.

1st Operation. Cæcostomy, resection of the neoplasm, colostomy.

2nd Operation. Lateral anastomosis between transverse colon and rectum.

Vaccine and nuclein treatment before 2nd operation.

John J., æt. 64. Had suffered from constipation, flatulence and pains in the abdomen for two months. Acute symptoms for one week.

State on Admission. Patient suffering from profound intestinal toxæmia. Abdomen greatly distended.

Operation, February 27th, 1909. Incision as for Inguinal colotomy, tumour of the fixed iliac colon discovered. During manipulation the tumour tore across, necessitating immediate resection. Open ends of colon brought out in left lumbar region. Incision made over cæcum and a cæcostomy performed. Patient survived this operation and bowels acted well through both openings. On March 5th, his opsonic index for B. Coli =·83. He was given a subcutaneous injection of 200 million B. Coli Vaccine.

On March 11th. Index for B. Coli = 1·13.

On March 16th. Index for B. Coli = 1·14. Given a second injection of 200 million B. Coli vaccine.

March 17th, 8 p.m. Leucocytes 6,500. Given a subcutaneous injection of 4 cc. of a 2 per cent. solution of nucleic acid.

March 18th, 10.30 a.m. Leucocytes 13,000. Opsonic Index for B. Coli = 1·62.

11 a.m. *Second operation.* Abdomen opened in mid line. The transverse colon and the pelvic colon were both cut across, all four ends closed and a lateral anastomosis between the transverse colon and the rectum performed. Patient never gave any cause for anxiety after this operation and made a rapid recovery.

Conclusions.

1. In peritoneal infections after intestinal operations the Bacillus coli communis plays a leading rôle.

2. Recovery from such infections depends on the patient's capacity and particularly on the capacity of his peritoneum to react promptly.

3. The presence of a leucocytosis in the blood stream favours a prompt peritoneal reaction.

4. The preliminary subcutaneous injections of a suitable vaccine and of nucleic acid enhances an animal's powers of reaction to peritoneal infection.

5. Preliminary treatment with a combination of vaccine and nucleic acid is of much greater value than treatment with either of these agents alone.

6. A vaccine made from a virulent culture is more efficacious than one made from an old stock culture of the same germ.

7. Two-stage operations for the removal of growths of the large intestine have many advantages, among others the fact that owing to the irritation caused by the first operation the peritoneum is in a measure prepared to withstand infection should it occur at the second operation.

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SERUM THERAPY IN ACUTE PERITONEAL INFECTIONS.[1]

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In the blood serum of an animal which has recovered from an acute infection we have good reason to believe that there are specific antibodies which have been produced in excess of the requirements of the animal. One might reasonably hope to beneficially influence the course of a similar infection in an animal by injecting into its blood stream some of the serum of another which had recovered.

The experiments recorded in this paper were designed to test whether in severe cases of acute peritonitis any benefit might be derived from the intravenous injection of the serum of a case which had recovered from a similar type of peritoneal infection. The experiments were carried out on rabbits, and to commence with *B. coli communis* was the organism employed to induce peritonitis. One very soon realised, however, that the peritonitis so induced was not comparable to that produced in the human being by this organism. In the rabbit which has succumbed to a *B. coli* peritonitis one finds, on opening the abdomen, some turbid fluid, great injection of the peritoneum, and possibly some flakes of lymph, but nothing resembling the purulent plastic type of peritonitis produced by this organism in the human subject. On making cultures from the heart blood of such a rabbit one gets, almost invariably, a very vigorous growth of *B. coli*, and it is evident that the animal died of a septicæmia. In the human subject dying from a peritonitis due to *B. coli* it is quite the exception for one to obtain a growth of this organism in cultures made from the patient's blood. In the acute peritonitis of man death is apparently due to toxæmia.

While working at this subject one had the good fortune to hit on a strain of *Staphylococcus aureus* which, after passage through several rabbits in large intravenous doses, produced, in reasonable dosage, a

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fatal peritonitis in those animals. This form of peritoneal infection is, I believe, much more comparable to human peritonitis than is the *B. coli* peritonitis of rabbits. Thus in eighty-five post-mortems on

TABLE I.

Animal used for Experiment.	Dose and Nature of Organism injected intraperitoneally.	Time allowed to elapse between the Induction of Peritonitis and the Injection of Immune Serum.	Dose of Immune Serum injected.	Time since the Inoculation of Recovered Animal from which Serum was obtained.	Result of Experiment.
EXPERIMENT 1—					
Rabbit (1) . . .	2000 million *Staphylococcus aureus*	6 hours	4 c.c.	7 days	Recovery.
Rabbit (2), control	,,	...	...	. .	Death in 30 hours.
EXPERIMENT 2—					
Rabbit (1) . . .	2500 million *Staphylococcus aureus*	5½ hours	4·5 c.c.	2½ days	Recovery.
Rabbit (2), control	,,	...	...	...	Death in 30 hours.
EXPERIMENT 3—					
Rabbit (1) . . .	2500 million *Staphylococcus aureus*	3½ hours	4 c.c.	5 days	Recovery.
Rabbit (2), control	,,	...	...	...	Death in 5 days.
EXPERIMENT 4—					
Rabbit (1) . . .	4000 million *Staphylococcus aureus*	1 hour	3·5 c.c.	2 days	Death within 16 hours.
Rabbit (2), control	,,	...	...	...	,,
EXPERIMENT 5—					
Rabbit (1) . . .	3000 million *Staphylococcus aureus* + 50 million *Pneumococcus*	8½ hours	7 c.c.	3 days	Recovery.
Rabbit (2), control	,,	...	...	...	Death in 30 hours.
EXPERIMENT 6—					
Rabbit (1) . . .	2500 million *Staphylococcus aureus* + 50 million *Pneumococcus*	7½ hours	4·5 c.c.	4 days	Recovery.
Rabbit (2), control	,,	...	...	...	Death in 2[illegible] hours.

rabbits dying of *Staphylococcal* peritonitis, cultures from the heart-blood remained sterile in thirty-seven cases; in eleven cases only one or two colonies of *Staphylococcus aureus* developed, and in nine cases a fairly vigorous growth took place in the tubes. In the latter cases the

animals had succumbed very quickly after a large intraperitoneal injection.

The local reaction in the peritoneal cavity in the staphylococcal cases was purulent, with flakes of lymph, and in many cases closely resembled the human type of peritonitis. Staphylococcal infections in the rabbit appear, therefore, to resemble the *B. coli* infections in the human subject, and *vice versa*.

Technique of experiments.—Having determined, approximately, the lethal dose of the bacterium to be used, two rabbits were each given an intraperitoneal injection of slightly more than the lethal dose of this bacterium. At the same time, from 10 to 20 c.c. of blood was drawn off into a test tube, with full aseptic precautions, from the auricular vein of a third rabbit which had just recovered from a peritonitis due to the same bacterium. This test-tubeful of blood was then put in the incubator at 37° C. to favour rapid clotting and separation of the serum. Several hours ($2\frac{1}{2}$ to 9 hours) later, when peritonitis was established in the two rabbits, the serum obtained from the blood of the recovered animal was injected into the auricular vein of one of the rabbits which had peritonitis. The course of the peritonitis and the fate of the animal was then carefully observed in each case.

The amount of serum injected varied from $2\frac{1}{2}$ c.c. to 7 c.c. in the different experiments, and it was always fresh, *i.e.* it was always obtained from a living animal a few hours before; and lastly, it was never allowed to cool, being kept in the incubator at 37° C. from just after its withdrawal from the recovered animal till just before its injection into the animal with peritonitis. (The two latter points I believe to be of importance.)

The bacteria used in these experiments were *B. coli communis* (Table II.), *Staphylococcus aurèus*, and a combination of *Staphylococcus aureus* and *Pneumococcus* (Table I.). This combination of cocci was used because one had accidentally found that it produced in rabbits a purulent plastic type of peritonitis resembling closely that seen in the human subject.

In the four experiments in Table II. *B. coli communis* was the organism used. The object of this set of experiments was to test whether the intravenous administration of the serum of a recovered animal was as effective in promoting recovery in *a type of peritonitis where septicæmia is the rule* as it was shown to be in one where septicæmia is the exception.

Experiments 8, 9, and 10 were designed to test also at what time the serum of the recovered animal is most effectively protective, *e.g.* whether the serum of an animal which has recovered from a peritonitis induced only two days before the serum was withdrawn was as effectively protective as the serum taken from an animal a week or more after recovery from peritoneal infection.

From these experiments one concluded that the serum treatment was not so helpful in rabbits for *B. coli* as for the staphylococcal infections, and therefore that its action was probably more antitoxic than antibacterial, and thus all the more suitable for use in human peritoneal infections. One could not say that the blood serum of the recovered animal was richer in anti-bodies just after recovery from

the symptoms of peritonitis than it was a week or more later. The evidence on this point, however, was by no means conclusive.

TABLE II.

Animal used for Experiment.	Dose and Nature of Organism injected intraperitoneally.	Time allowed to elapse between the Induction of Peritonitis and the Injection of Immune Serum.	Dose of Immune Serum injected.	Time since the Inoculation of Recovered Animal from which Serum was obtained.	Result of Experiment.
EXPERIMENT 7—					
Rabbit (1) . . .	2500 million *B. coli*	4 hours	6 c.c.	2 days	Death 24 hours later.
Rabbit (2), control	,,	...	...	...	Death 12 hours later.
EXPERIMENT 8—					
Rabbit (1) . . .	800 million *B. coli*	3 hours	2·5 c.c.	4 days	Recovery prompt.
Rabbit (2) . . .	,,	,,	5 c.c.	11 days	,,
Rabbit (3), control	,,	...	...	...	Very ill, but recovered.
EXPERIMENT 9—					
Rabbit (1) . . .	1000 million *B. coli*	6 hours	5 c.c.	2 days	Recovery.
Rabbit (2) . . .	,,	,,	,,	6 days	Death 24 hours later.
Rabbit (3), control	,,	...	...	...	Death 19 hours later.
EXPERIMENT 10—					
Rabbit (1) . . .	1800 million *B. coli*	3 hours	5 c.c.	5 days	Death 12 hours later.
Rabbit (2) . . .	,,	,,	,,	7 days	Recovery prompt.
Rabbit (3), control	,,	...	...	...	Ill, but recovered.

It had previously been shown by Loeffler and Abel that the injection of the serum of a normal animal into the blood stream of one suffering from an acute infection gives little or no protection. To confirm this point, however, the experiment given in Table III. was carried out.

If we take the two first mentioned types of peritonitis we see that in the animals receiving the anti-serum (see Table IV.) the death rate was but 10 per cent., whilst in the animals not treated with anti-serum the death rate was 80 per cent. These facts are sufficiently striking to warrant one in adopting this method of treatment in bad cases of peritonitis in the human subject.

TABLE III.

Animal used for Experiment.	Dose and Nature of Organism injected intraperitoneally.	Time allowed to elapse between the Induction of Peritonitis and the Injection of Immune Serum.	Dose of Immune Serum injected.	Time since the Inoculation of Recovered Animal from which Serum was obtained.	Result of Experiment.
EXPERIMENT 11—					
Rabbit (1) . .	2500 million *Staphylococcus aureus*	4 hours	5·5 c.c.	3 days	Recovery.
Rabbit (2) . .	,,	,,	5 c.c.	Normal rabbit serum	Death 26 hours later.
Rabbit (3), control	,,	...	...	...	Death 18½ hours later.

TABLE IV.—*Summary of Results of Serum Therapy in Peritoneal Infections of Rabbits.*

	Nature of Peritonitis.	Number of Cases.	Recoveries.	Deaths.
Serum	*Staphylococcal*	6	5	1
No serum	,,	6	0	6
Serum	Mixed *Staphylococcal* and *Pneumococcal*	4	4	0
No serum	,,	4	2	2
Serum	*B. coli*	6	4	2
No serum	,,	3	2	1

EFFECT OF HEATING AT 57° C. FOR HALF AN HOUR ON THE PROTECTIVE PROPERTIES OF THE SERUM OF THE RECOVERED ANIMAL.

One now wished to know whether the serum would be as effective in its protective power were it kept for some length of time, and whether one could thus keep a stock of sera for use in different types of peritoneal infection. If none of the protective properties were lost on heating for 30 minutes at 57° C. then it was probable that the sera would retain their value though kept *in vitro* for some time. This would naturally allow of a much wider clinical application of the method than if only fresh serum could be used.

The following experiments were accordingly carried out (Table V.).

TABLE V.

Animal used for Experiment.	Dose and Nature of Organism injected intraperitoneally.	Time allowed to elapse between the Induction of Peritonitis and the Injection of Immune Serum.	Dose of Immune Serum Injected.	Time since the Inoculation of Recovered Animal from which Serum was obtained.	Result of Experiment.
EXPERIMENT 12—					
Rabbit (1) . .	3000 million *Staphylococcus aureus*	6½ hours	4 c.c.	2 days	Recovery.
Rabbit (2) . .	,,	,,	,,	H. S., 2 days	Death 14 hours later.
Rabbit (3), control	,,	...	...	...	Death 19 hours later.
EXPERIMENT 13—					
Rabbit (1) . .	2000 million *Staphylococcus aureus*	3 hours	4 c.c.	8 days	Recovery.
Rabbit (2) . .	,,	,,	3·5 c.c.	H. S., 8 days	Death 30 hours later.
Rabbit (3), control	,,	...	...	...	Ill, but recovered.
EXPERIMENT 14—					
Rabbit (1) . .	2000 million *Staphylococcus aureus*	6 hours	4·5 c.c.	6 days	Recovery.
Rabbit (2) . .	,,	,,	,,	H. S., 6 days	Died 2½ days later.
Rabbit (3), control	,,	...	...	...	Ill. but recovered.

These experiments showed that by *heating the serum its protective properties are destroyed*, and that instead of being beneficial it was apparently harmful when given to an animal with peritonitis.

They indicate, moreover, that Wright's contention that any beneficial effect that an anti-serum may have lies in the fact that it is really a type of vaccine is unjustifiable, for heating is an essential in the preparation of any vaccine, and does not destroy its therapeutic value.

It would seem evident, then, that for an anti-serum of this class to be of value it is essential that it be fresh and unheated at the time of injection.

CLINICAL APPLICATION IN MAN.

One has had the opportunity to apply serum treatment clinically to six human cases of peritonitis. One naturally selected only the

severest cases of peritoneal infection, and in no case was this treatment commenced until the worst prognosis had been given and it was thought that all the methods of treatment generally employed in such cases would not avail to save the patient. All the cases treated had undergone a surgical operation for their complaint some hours or days before the serum treatment was commenced. Needless to say, all other treatment was not stopped when the serum therapy was begun, it being only used as an adjuvant; thus one could not dogmatise but merely speculate as to what credit or discredit belonged to the serum injections.

On the other hand, by carefully watching these cases one could note how the hourly course of the signs and symptoms of the patients treated was influenced by the serum injection, and particularly how their "General Condition" was affected by this form of treatment.

General Method of Application of, and Technique employed in, Serum Treatment.—From film preparations and cultures made at the time of operation the nature of the peritoneal infection was ascertained. If the case were doing badly and recovery was considered unlikely, another case was sought for in which a similar type of peritoneal infection had been successfully treated and in which recovery was assured. In the wards of an hospital of any size there could very seldom be any difficulty in finding such a case. The next step was to make sure that the recovering patient did not suffer from specific or other infectious disease, and then to obtain his or her permission to withdraw a quantity of blood from a vein. (In no case was this permission refused me.) Having obtained the consent of the patient, one sterilised the front of the elbow with alcohol and ether, and using a sterile needle and syringe withdrew from 20 to 70 c.c. of blood from the median basilic vein. The blood was put into four sterile test tubes which were plugged and left in the incubator at 37° C. for from 3 to 6 hours. At the end of this time the serum which had separated was taken up in a sterile syringe and immediately injected with aseptic precautions into the median basilic vein of the patient whose life was in danger. The serum was thus never allowed to cool between withdrawal and injection, being regarded as a living tissue and treated as such. Five of the six cases treated received more than one injection and (with one exception) the serum was obtained fresh, as above described, for each injection.

As will be seen from the description of the cases which followed, various types of pure and mixed infections were treated with corresponding sera. The size of the dose was only limited by the amount of serum obtainable, though from the work of Loeffler and Abel one inferred that a large quantity of serum is not essential.

Case 1.—Acute Suppurative Appendicitis.—Boy, J. M., æt. 12. Admitted to hospital on 19th November 1908. Six days before admission he was seized with sudden abdominal pain and vomiting, both of which symptoms

persisted with slight intermissions until his removal to hospital. Had not passed flatus for two days and had emaciated greatly.

State on admission.—Child obviously very ill, face pinched, eyes sunken; tongue dry and brown; pulse, 110 feeble. General tumidity of the abdomen, which does not move with respiration. Marked tenderness in right flank and all over the lower half of the abdomen.

Operation.—1 p.m. two hours after admission.

A large abscess filling up the right iliac and lumbar regions of his abdomen was opened, a gangrenous perforated appendix removed, and through a suprapubic wound a large collection of foul smelling pus escaped from the pelvis. A film made from the pus showed degenerated pus cells and swarms of a colon-like bacillus, also a few chains of streptococcus. The abscess cavities were douched out and drained.

At 7 p.m. the same evening the child was very ill. Pulse, 124, very small; respiration, 56, hands cold. A very bad prognosis was given.

Accordingly 65 c.c. of blood was withdrawn from the median basilic vein of a Mrs. M. who had recovered (20 days) from an acute suppurative appendicular peritonitis due to *B. coli*. The blood was put in the incubator, and at midnight 21 c.c. of the blood serum which had separated out was injected into the median basilic vein of the boy. Next morning the child looked much better, though the pulse was still 120. The following day pulse was 108, and it came down steadily, the child leaving hospital, cured, at the end of four weeks.

The improvement in this boy's general condition some hours after giving the serum was very striking, and one had no doubt that great help had been given by its injection.

Case 2.—Streptococcal Peritonitis (Appendicular).—D. H., young, man, æt. 19. Admitted to hospital on 4th January 1909. Two days before admission patient was suddenly seized with acute abdominal pain and vomiting. Pain became steadily more severe till admission. Had no sleep at night, and vomited everything given him by mouth. Had passed no flatus for 36 hours.

State on admission.—Powerful young man, flushed and breathing rapidly, tongue furred and rather dry. Pulse, 110; temperature, 101; respiration, 38. General tumidity of the abdomen; abdominal movement greatly restricted, absent below umbilicus. Marked tenderness in right iliac region and in suprapubic region.

Operation.—One hour after admission. Mesial incision below umbilicus; tubes introduced into the pelvis, from which turbid sero-pus escaped; pelvis washed out. Gridiron appendix incision now made, a gangrenous perforated appendix found lying in a partially walled-off abscess in the right iliac fossa. Iliac fossa and pelvis drained. Film made from pus at operation showed presence of many chains of streptococci, and also a colon-like bacillus.

The following morning, pulse, 108; respirations, 38, but not looking well. Patient got steadily worse during the day, and at 10 p.m. his pulse was 140 and a very bad prognosis was given.

Thirty c.c. of blood was obtained in the usual manner from a patient, J. W., operated on 8 days before for suppurative appendicitis due to *B. coli* and streptococcus, and who was doing well. At 1 a.m. on 5th January, 11 c.c. of serum obtained from this blood was injected into the median basilic vein of D. H.

Next morning patient had improved, but his abdomen was still much distended and his pulse 124. Cultures made at the operation now showed the infection to be almost purely streptococcal, only three or four colonies of *B. coli* having grown, compared with several hundred colonies of the streptococcus. On the following day, 6th January, patient was still very ill. Pulse, 120; respirations, 38. Accordingly, 30 c.c. of blood was withdrawn from the

arm of J. S., who had been operated on two and a half days before for a localised pure streptococcal suppurative peritonitis due to appendicitis, and who was now out of danger. At 6.30 p.m. on 6th January 10 c.c. of serum obtained from this blood was injected into the cephalic vein of D. H.

Next day patient was much improved, his tongue was moist, his pulse rate had fallen, and thereafter he made an uninterrupted recovery.

Case 3.—Suppurative Appendicitis (Streptococcal).—R. C., young man, æt. 19. Admitted to hospital on 25th January 1909. For eleven days previously had felt out of sorts, had intermittent abdominal pain, and had vomited occasionally. For the three days before admission had been seriously ill with pain all over the lower abdomen and persistent vomiting, the vomit containing altered blood.

State on admission.—Thin and pale young man, eyes sunken, tongue dry. Pulse only 78; temperature, 98·4. Leucocytes, 20,000. Glycogen reaction, +. Lower half of the abdomen is tumid and is rigid. Marked suprapubic tenderness; also tenderness in both iliac regions.

Operation.—One hour after admission. Usual gridiron appendix incision. A large swelling, resembling in appearance an ovarian cyst, was seen rising out of the pelvis and lifting up the rectum. A fresh incision, five inches long, was now made in the mid line below the umbilicus, and then, to gain still better access, the two incisions were joined by cutting across the right rectus muscle transversely. The peritoneal cavity having been thoroughly packed off with gauze, the swelling was opened, and over 10 oz. of thick pus escaped, and a gangrenous appendix removed. The cavity was swabbed out, and drained through the lower end of the suprapubic wound. The other wounds were stitched up. Cultures made from the pus gave a very vigorous and practically pure growth of a streptococcus, only three colonies of *B. coli* developing.

For the first two days, though the boy looked far from well, his pulse and temperature remained practically normal. On the third night after operation, however, his pulse rose to 130; temperature, 102·4; there was tenderness over the lower half of the abdomen, and he looked very ill. Accordingly, 20 c.c. of blood was obtained from the previous case, D. H., who had now recovered, and 4 c.c. of serum from this was injected into his median cephalic vein. Next morning he had improved considerably, but in the evening was again very ill. Thirty c.c. of blood was drawn from the arm of the patient, J. S. (pure streptococcal case), and 12 c.c. of the serum from this was injected intravenously. The following morning temperature and pulse had fallen, and patient looked better. At the dressing that forenoon a streptococcal abscess in the abdominal wall was opened, and thereafter pulse and temperature fell to normal.

Patient now did well for two days, but on 3rd February pulse and temperature again ran up, and he appeared to be in a very critical condition. Again 30 c.c. of blood was obtained from patient, D. H., and 9 c.c. of the serum which separated was given him intravenously. Next day pulse and temperature had fallen, the patient looked very much better, and from that time onwards made a perfect recovery.

Case 4.—Streptococcal Peritonitis (Appendicular).—F. G., boy, æt. 12. Admitted to hospital on 15th February 1909. History of abdominal pain and vomiting for three days before admission. Pain on passing water, and no passage of flatus for two days.

State on admission.—Patient lying with his legs drawn up, face drawn and pinched, eyes sunken, tongue dry. Pulse, 98; temperature, 99·4. Leucocytes,

16,000. Glycogen reaction, +. General tumidity of the abdomen, which is fixed in its lower half. Pain and tenderness most marked over appendix region, but present over the whole lower half of abdomen.

Operation.—One hour after admission. Gridiron appendix incision. Clear serum escaped on opening abdomen. A retrocæcal abscess was opened, and in it a gangrenous and perforated appendix was found and removed. Cavity swabbed out and drained. Film of pus made at operation showed pus cells and diplococci in chains.

The following day the boy looked very ill. Pulse, 120, and persistent vomiting.

Cultures made at operation now showed a growth of small colonies, films from which revealed chains of diplococci resembling pneumococcus. (An expert bacteriologist was consulted, and he was also of the opinion that it was a pneumococcal infection. To make certain, subcultures were made in different media, and a rabbit was inoculated.) Acting on this evidence, 20 c.c. of blood was withdrawn from the arm of J. P., a case of pure pneumococcal peritonitis due to appendicitis, who had been operated on eight days before, and who was rapidly recovering. At 7 p.m. on 16th February 6 c.c. of serum from this blood was injected into the boy's median basilic vein.

The following morning there was little improvement in his condition, and his vomiting continued. It was now found from the subcultures that the organism was much more like a streptococcus than a pneumococcus, the diplococcal forms having disappeared. The rabbit appeared none the worse for its injection, which was also in favour of the organism being streptococcus. However, as some doubt still existed, it was thought advisable to give the boy both types of serum. Therefore 30 c.c. of blood was got from J. P. (pneumococcal) and 20 c.c. from R. S. (Case 3, streptococcal), and 12 c.c. of serum obtained from the former, along with 6 c.c. of serum from the latter, injected intravenously into boy at 5.30 p.m. on 17th February. Next morning (18th) the boy had improved considerably; the pulse had fallen to 110, and he looked better in every way. Towards evening again, however, he relapsed somewhat. From the cultural and inoculation tests it was now quite certain that the infection was streptococcal in nature.

Once more 30 c.c. of blood was taken from the arm of R. S. (Case 3), and at 6.30 p.m. on 18th 11 c.c. of serum from this was injected intravenously into the boy. Great improvement followed, and next morning the pulse was 96, tongue was cleaner, and the facies had lost the drawn expression. In the evening the improvement was maintained; pulse, 90. On the morning of the 20th, however, the boy had sudden acute pain in the abdomen, the pulse and temperature rapidly ran up, he became collapsed, and he died at 6.30 p.m. the same day. Unfortunately, a sectio could not be obtained; but, so far as one could judge, some localised collection of pus must have ruptured into his general peritoneal cavity.

In this case, perhaps more than in any other, was one impressed by the marked diminution in the toxæmic symptoms which resulted from the injection of a suitable serum, and it was particularly unfortunate that one could not ascertain exactly what was the sudden catastrophe which carried off the patient.

Case 5.—General Peritonitis (*B. coli*) after Pylorectomy.—Mrs. D., æt. 64. On 14th January pylorectomy performed for gastric carcinoma. On the second day there was a rise of temperature, which was maintained on the third day. Suddenly, on the third night after operation, patient was seized with sudden acute pain generalised over the abdomen, and became collapsed. General peritonitis was diagnosed, and under chloroform the abdomen was

freely opened in the mid line. It was found that a localised collection of pus had taken place round the duodenal stump, and this had ruptured into the general peritoneal cavity, which contained sero-purulent fluid. Abdomen washed out and drained. *B. coli* was grown on culture from the pus in the abdomen.

The following day (17th) patient was very ill, and required intravenous infusion of saline. On the 18th pulse was very feeble, and patient was only semi-conscious. Fifty-six c.c. of blood obtained from the arm of Helen L., who was recovering (nine days) from an acute suppurative *B. coli* appendicitis; 18 c.c. of serum from this was injected into the patient's median basilic vein. A few hours later a distinct improvement was noted in the patient's pulse and in her general condition, and the improvement was maintained for ten hours. Patient, however, still continued to have a high temperature, and as no flatus was passed several loops of small intestine were opened and washed through. On the 20th the patient was again unconscious, and her pulse very weak. Other 60 c.c. of blood was obtained from Helen L. (*B. coli*, appendicitis case), and 20 c.c. of the serum from this was given intravenously, along with a pint of saline. There was no immediate improvement in the pulse, but six hours later a great change for the better was visible in the patient's condition. Her pulse became slower and stronger, and she regained consciousness.

On the 21st she again relapsed. Other 4 c.c. serum given along with 1 pint of saline; this time, however, without much benefit. The patient gradually sank, and died ten days after the onset of peritonitis.

Post-mortem.—A subphrenic abscess, surrounding the spleen, and containing over a pint of foul pus, was found.

In this case again improvement followed the use of serum, and a very definite organic cause, which, of course, one could not hope to influence by serum treatment, was found to account for death.

Case 6.—General Peritonitis (*B. coli*) Appendicular.—J. P., boy, æt. 13. Admitted to hospital on 15th March 1909. History of abdominal pain and vomiting for the past five days, and during the past two days gradually increasing distension of the abdomen.

State on admission.—Boy very ill, face pinched, tongue dry. Pulse, 124, and very small; temperature, 100. Abdomen tumid, no movement, with respiration in its lower half. Marked tenderness all over lower abdomen and in right loin. Leucocytes, 20,000. Glycogen reaction.

Operation.—Half an hour after admission. Usual appendix incision; localised abscess in right iliac fossa opened; foul pus escaped. Suprapubic opening made; pelvis found full of pus; washed out and drained. The iliac incision was enlarged upwards and outwards, and a large retrocæcal abscess opened, and in this a gangrenous and perforated appendix was found and removed. Free drainage.

The next day (16th) pulse 124, vomiting frequently, and abdomen distended. On the 17th vomiting continued, and the abdomen was more distended. A general anæsthetic was given, the abdomen opened in the mid line, and five coils of small intestine were opened, tubes inserted and stitched in, and the coils of intestine washed through with saline. Was pulseless at conclusion of operation, and required transfusion on the table.

Twenty c.c. of blood obtained from the arm of W. C., who had been operated on nine days previously for acute suppurative *B. coli* appendicular peritonitis, and who was now recovering. At 7 p.m. the boy's pulse was 160, and he was restless and cold. 6·5 c.c. of W. C.'s blood serum was injected into his median cephalic vein.

The boy improved during the night, and the next morning was a little

better. At 2 p.m. on the 18th, however, he became much worse. Other 30 c.c. of blood obtained from W. C., and at 6.30 p.m. 12 c.c. of this injected into vein of the boy. He was practically moribund by this time, however, and died two hours later.

Post-mortem.—A large subphrenic abscess was found surrounding the spleen (evidently four to five days old), another abscess under the liver, and a third in the left iliac and lumbar regions.

The results obtained in these six cases were very encouraging, and convinced one that this form of treatment is of considerable value in peritoneal infections of the human subject.

The proportion of streptococcal cases is merely a coincidence, and does not, I think, represent the proportional incidence of that organism in severe cases of peritoneal infection.

One has not been able to find any previous record of the use of human immune serum in the treatment of peritonitis.

Loeffler and Abel (1896), experimenting with the guinea-pig, found that a dose of 0·3 c.c. of the serum of a dog immunised against *B. coli* gave a guinea-pig protection against from twenty to fifty times the lethal dose of *B. coli* injected into its peritoneal cavity.

Makin and Sargent (1907) have employed a polyvalent coli serum, obtained from the horse, in the treatment of severe cases of peritonitis. The results obtained from its use, however, though in some respects encouraging, were by no means striking.

Recently Mackenzie and Martin (1908) have obtained good results from the injection of the blood serum of patients who had recovered from cerebro-spinal meningitis into the spinal canal of patients in the acute and critical stage of that disease.

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OBSERVATIONS ON THE MORBID HISTOLOGY AND BLOOD CHANGES IN MYELOCYTHÆMIA.[1]

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I. Preliminary.

In a recent paper (1906) we discussed the pathology of lymphatic leukæmia and chloroma.

We now propose to give an account of the histological changes found in seven cases, with brief notes of the clinical features of the first case.

We have also tabulated the results of enumeration and differential counts of the leucocytes in twenty-four cases.

The methods employed were similar to those used in our research on lymphæmia.

II. Cases.

Case 1.

Clinical Notes.—Man, æt. 40, unmarried, a boatman, began to suffer from breathlessness, and ten months before coming under observation noticed an abdominal tumour. On two occasions within six weeks of admission he suddenly developed a large hæmatoma in the back. Family history negative. No history of syphilis or alcoholism. On examination the liver and spleen were found to be greatly enlarged, the latter reaching Poupart's ligament. The cervical, axillary, and inguinal glands were slightly enlarged. There was œdema of the feet and legs. Under X-ray treatment some improvement occurred. The blood condition improved and the spleen diminished in size. The liver remained as large as ever. Patient was able to leave hospital, but returned worse in a few weeks and died.

In making differential counts some peculiar cells in addition to those corresponding to the ordinary types were noticed.

(*a*) Many of the myelocytes had very scanty granulation.

(*b*) A very large cell closely resembling the large lymphocyte, showing a large pale nucleus and deeply stained finely granular protoplasm, was present in large numbers in the later counts.

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[2] Received October 30, 1907.

(*c*) In a few instances the latter cells showed a scanty basophile (azure) granulation. Such cells could be distinguished from the myelocytes by the more basophile reaction of the granules and the deep basophile staining of the cytoplasm. Mitosis was noticed in some of these cells.

Blood Examination.

	Red Blood Corpuscles.	White Blood Corpuscles.	Hæmoglobin.
August 20, 1905 . .	2,670,000	451,875	40 per cent.
September 24, 1905 . .	2,720,000	285,625	40 ,,

(*d*) In many of the forms the centrosomes were large and prominent. While in the following table these cells have been separately classed (lymphoid cells), there were gradations between the basophile "lymphoid" cell, with or without granules, and the typical large lymphocytes. We therefore regard them as merely slight departures from the ordinary form of the typical large lymphocyte.

Date.	Polymorphs.	Myelocytes.	Eosinophiles.	Eosinophilic Myelocytes.	Small Lymphocytes.	Lymphoid Cells.	Large Lymphocytes.	Basophiles.	Per 1000 Leucocytes.	
									Megaloblasts.	Normoblasts.
Aug. 22 . .	18	68	2	1	1	2	2	7	0	0
Sept. 24 . .	19·6	44	3	1	0·4	3	22	7	0	64
,, 28 . .	31	46	1	0·4	1	1	18	1·6	0	12
Oct. 11 . .	7	58	0·2	0	0·2	0·6	34	0·6	16	16
Marrow . .	2	47	1	4	0	0·5	45	0·5	0	15

The section revealed great enlargement of the lymph glands and spleen. The glands were firm, and felt almost gritty on cutting.

Histology.—*Stomach.*—A good deal of post-mortem digestion. No gross leucocyte infiltration. Epithelium shed, except at bottom of crypts. Leucocytes not more numerous than normal in the mucous membrane. Those present were mainly lymphocytes and a fair number of eosinophiles. Other coats normal. No pigmentary changes. A piece of adhering omentum showed great infiltration, with leucocytes between the fat cells. The varieties present were lymphocytes, eosinophiles, basophiles, and eosinophile myelocytes. The great bulk was composed of lymphocytes.

Small intestine.—Post-mortem digestion. Much of epithelium shed. Very few leucocytes in coats. Cells in Peyer's patch comparatively scanty. The cells there were ordinary lymphocytes. In the submucosa, especially under the Peyer's patches, were numerous cells rather irregular in shape, with a small darkly stained round nucleus and large rather darkly stained eosinophile granules. The bowel content consisted mainly of shed epithelium, leucocytes,

and some organisms, with granular detritus. Many of the leucocytes were eosinophiles similar to those seen in the submucosa. No pigment was seen.

Pancreas.—A few leucocytes, mainly polymorphs, were scattered between the acini. No special aggregations.

Liver.—The whole of the lobules were broken up, and the columns of cells were squeezed into thin strands or had altogether disappeared. In a few lobules there were a few comparatively unaltered cells round the intralobular vein, which was packed with leucocytes, while the portal veins were comparatively empty. In some parts the destruction of liver cells had given rise to a fibrous change. Where the infiltration was massive there were very large blood spaces and round them masses of leucocytes, separated by strands of fibrous tissue, recalling the structure of an alveolar sarcoma. In some of these blood spaces were large giant cells with basket-shaped nuclei, and with the differentation of the protoplasm into an outer and inner zone which is found in the giant cells of bone narrow. The portal spaces were practically free from leucocyte invasion. In the blood spaces and surrounding liver tissue the cells were mainly lymphocytes and neutrophile myelocytes with some nucleated red cells. A considerable number of cells showed mitotic figures. Passing away from these blood spaces among the liver cells more eosinophiles of both types appeared. Some of the polymorphs had markedly branched nuclei (karyorrhexis. There were very numerous phagocytes in these spaces; in some parts six or eight in one field of a $\frac{1}{12}$ oil immersion lens. In the liver cells was a fair amount of pigment. In some parts a considerable amount of this gave the iron reaction, but the incidence of this change was very partial. Among the leucocytes were masses of pigment in leucocytes and endothelium. A curious appearance noted was the presence of pigment in a cell showing a monster mitotic figure. Here and there this pigment gave the iron reaction.

Spleen.—Malpighian bodies small and indefinite. The great mass of the pulp is taken up with red blood corpuscles. Leucocytes are scanty, even as compared with a normal spleen. Eosinophiles of both varieties were present in islands around the remains of the Malpighian corpuscles. A few basophiles were seen. There were some myelocytes and a good many nucleated red cells. Polymorphs were scanty. Phagocytes were seen in fair numbers. Mitotic figures were fairly common. There was a great deal of golden brown pigment both free and in cells, leucocytes, and endothelium. This gave the iron reaction in scattered areas, notably in cells along the trabeculæ and along the connective tissue fibrils. The iron pigment was often in large masses.

Kidneys.—Comparatively little affected. Infiltration principally situated round the interlobular arteries and round some of the glomeruli. There was a less amount in the medulla. Cells of the convoluted tubules and the glomeruli were normal. No pigment was seen. Parts of the organs were very vascular.

Suprarenals.—Vessels distended, but no infiltration between the gland cells. Here and there in the fat outside was some leucocyte infiltration, as seen in the omentum. No pigmentary changes.

Heart.—Vessels distended with leucocytes.

Lungs.—Vessels distended with leucocytes. Masses of black pigment and a few small granules of iron pigment were seen.

Thyroid.—Practically normal. No pigment was found.

Lymphatic glands.—Structure lost. Here and there were islands of small lymphocytes which had no resemblance to germ centres. Between these islands were cell areas composed of eosinophiles, eosinophile and neutrophile myelocytes, and in some cases these were inextricably mixed up with small lymphocytes. The subcapsular sinus was normal. No iron. In different glands the proportion of lymphocyte to granular areas differed, but there was no essential difference. A few lymphocytes showed karyorrhexis.

Hæmolymph glands.—These were unusually large, and showed the same general arrangement as the lymph glands, with the addition of red cells strewn

through the spaces. Few phagocytes ingesting cells. Some mitotic figures. A few "mulberry" cells. There was a great deal of pigment, often in large masses, lying both in cells and free. A large proportion of this gave the free iron reaction.

Thymus.—Only a few lymphocytes in fat and fibrous tissue remained.

Bone marrow.—Fat had entirely disappeared. The density of the cellular arrangement differed from place to place. There were more white cells and fewer red cells than normal. Many of the red cells were nucleated, and most of the nuclei showed curiously branched processes (karyorrhexis). Some showed polychromasia. The bulk of the white cells were myelocytes, but all other varieties were represented; many of the polymorphs showing the branching nuclear change already referred to. Giant cells were few, small, and degenerated. Phagocytes ingesting cells were present in fair numbers. Very little pigment was seen. It was mostly contained in the endothelium of the blood spaces, and did not give the iron reaction. A differential count of the marrow cells is given along with the blood counts.

CASE 2.—A Man, æt. 31 (Case 15 on p. 224).

HISTOLOGY.—*Stomach.*—Mucous membrane congested. In deeper coats were a few collections of lymphocytes.

Small intestine.—Villi digested. Epithelium well preserved in Lieberkühn's follicles. There was an excess of leucocytes in the submucosa, and there were scattered white cells in the muscular coat. The cells were lymphocytes and eosinophiles.

Pancreas.—Much digested. Vessels packed with leucocytes.

Liver.—There was fatty degeneration, especially at the periphery of the lobules. There were deposits of leucocytes in the portal spaces, and the capillaries were dilated and engorged with leucocytes, in some cases right up to the centre of the lobule. In addition to polymorphs, eosinophile and neutrophile myelocytes, eosinophiles and large lymphocytes, there were numerous large phagocytes enclosing leucocytes and red cells. A few nucleated red cells were noted. Some of the endothelium of the capillaries was greatly swollen at certain points, and contained a large amount of pigment, which gave the iron reaction. In the liver cells there was also iron pigment in very large amount at parts, but very irregularly distributed.

Spleen.—Malpighian bodies small. Pulp very congested, and contained leucocytes corresponding to those found in the blood, and some normoblasts.

Kidneys.—Congested. There were slight catarrhal changes and casts in some of the collecting tubules. There were islands of leucocytes between the medullary tubules, and in the cortex leucocyte infiltration had destroyed some of the tubules.

Suprarenals.—There were a few islands of leucocytes in the medulla, and extending along the capillaries in the cortex.

Heart muscle.—There were numerous small collections of white cells in the connective tissue between the fibres. These consisted of lymphocytes, polymorphs, and eosinophiles.

Lung.—There was fibrinous pneumonia. The cells in the exudation were polymorphs.

Cerebrum.—Normal. Vessels packed with myelocytes.

Tonsils.—Normal. Germ centres few. Lingual tonsils.—There were some germ centres in the cortex.

Lymphatic glands.—A large axillary gland showed lost structure and great vascularity. Only lymphocytes were seen. A mesenteric gland showed the usual structure with defined lymph paths. The cells were large and small lymphocytes, some eosinophiles, a few giant cells of bone-marrow type, and a few phagocytes in the lymph paths ingesting lymphocytes.

Bone marrow.—The fat had all disappeared. There were numerous neutrophile and eosinophile polymorphs and myelocytes. Red cells and lymphocytes were scanty. No giant cells were seen.

Case 3.—A Man, æt. 40.

Histology.—*Liver.*—There was great infiltration by leucocytes in the portal spaces and between the rows of liver cells. The majority of the cells were myelocytes—some in mitosis. There were some basophile myelocytes. The liver cells were pressed upon, and in many cases shrunken. There was a large amount of pigment in the liver cells and in the capillary endothelium. The endothelial cells were prominent, and projected into the blood stream. In the liver cells there was most pigment at the edge of the portal spaces, but it extended a considerable distance into the lobules. All the pigment gave the iron reaction.

Spleen.—There were no definite Malpighian corpuscles. The pulp was fibrous and the cells shrunken. There were numerous giant cells; as many as in normal marrow. Some showed degenerative changes. There was some iron pigment.

Kidneys.—There was very great leucocyte infiltration in rather discrete patches. In these patches kidney structure was destroyed. Where the tubules were present they were catarrhal and degenerated, and showed polymorph invasion. The Malpighian bodies were very fibrous. Between the layers of Bowman's capsule were numerous leucocytes. The collecting tubes were crowded together by masses of cells. There were casts consisting of granular débris. There were a fair number of giant cells in the connective tissue in the medulla. There was a fair amount of pigment in the endothelium.

Heart.—Marked brown atrophy. On the mitral valve was a fibrinous vegetation containing leucocytes and organisms.

Lung.—There were patches of catarrhal pneumonia with ordinary neutrophiles in the exudation. The capillaries were filled with leucocytes.

Lymph glands.—Distinction between cortex and medulla quite definite. Mitoses were numerous. There were many giant cells with basket nuclei lying among the lymphocytes with no red cells near. There were also large cells ingesting red corpuscles. There was some pigment here and there in the endothelium and in free cells, but none in very large masses.

Brain.—The vessels were distended with leucocytes. The lymphatic spaces seemed to contain more leucocytes than the veins, and the veins more than the arteries.

Case 4.—Man, æt. 50.

Small intestine.—There were some large lymphocytes and some large cells containing pigment in the fibrous tissue between the gland tubules, and also in the submucosa. The pigment did not give the iron reaction. The lymph follicles showed no enlargement. There were very numerous basophiles in the muscular coat.

Tongue and lingual tonsil.—Large number of basophiles in the lymphoid tissue and submucosa.

Pancreas.—There was no infiltration. The vessels and lymphatics were full of leucocytes.

Liver.—Numerous accumulations of leucocytes were seen in scattered areas. These were very extensive in the region of the portal spaces. There were some collections in the capillaries large enough to compress the liver cells. Many of the portal veins showed thrombosis. The cell infiltrations consisted mainly of myelocytes, but there were also numerous large lymphocytes, which in some instances preponderated. These latter were always found in clumps.

There were a few basophiles and some nucleated red cells. There were a few phagocytes ingesting leucocytes. No giant cells were seen. Many of the lymphocytes had peculiar branching nuclei. Many of the endothelial cells of the capillaries were swollen and prominent, and contained large masses of iron pigment. Iron was also present in free cells in the capillaries. There was very little pigment in the liver cells, and none of it gave the iron reaction.

Spleen.—Malpighian bodies not distinguishable. Trabeculæ obscured. Pulp was very cellular. There were areas where the red cells preponderated, but the great majority of the cells were leucocytes, mainly myelocytes and polymorphs. Lymphocytes were not so numerous as in the liver. There was a fair number of normoblasts and a few phagocytes. There were granules of iron pigment in large cells along the trabeculæ, and also in the pulp in the neighbourhood of trabeculæ. The endothelial cells of the sinuses were swollen and prominent, and also contained iron pigment.

Kidneys.—There were enormous deposits of white cells scattered irregularly. There were masses under the cortex displacing and destroying the convoluted tubules, and running down the pyramids of Ferrein. The tubules were catarrhal, and many of the convoluted and collecting tubules contained casts. Many of the Malpighian bodies were almost entirely converted into fibrous tissue. The infiltrating cells were myelocytes and polymorphs, a few eosinophiles and large lymphocytes. In a few isolated areas there were masses in the kidney tubules which stained a diffuse blue with the ferrocyanide and hydrochloric acid. There were also granules of iron along the fibrils of connective tissue.

Lymph glands.—The mesenteric glands showed thickening of the capsule and septa. The gland tissue consisted of discrete nodules. Germ centres were few. The cells in the glands were mainly small lymphocytes with an occasional large one, a few polymorphs, and eosinophiles. No myelocytes. There were a few cells with dendritic nuclei. The vessels were filled with white cells. The connective tissue contained many basophiles.

Thyroid.—On one part there was an aggregation of thyroid cells full of iron pigment. Throughout the gland were large cells in the intervesicular connective tissue containing iron pigment in large granules.

Cerebrum and cerebellum.—Vessels engorged with white cells. Nerve tissue was normal.

Marrow.—The fat had disappeared. The great bulk was composed of myelocytes. The capillaries were densely packed with these cells. Among the myelocytes were islands of red cells in which some megaloblasts showing marked polychromasia were seen. There were very few normoblasts. There were a few basophiles. Large phagocytes were numerous. Giant cells were few and degenerated. There was no pigment.

Case 5.—A Man, æt. 44, who had an enormously enlarged spleen. No enlargement of lymphatic glands.

Blood Condition.

	Red Cells.	Hæmoglobin per Cent.	Leucocytes.	Polymorphs.	Myelocytes.	Lymphocytes.	Eosinophiles.
Sept. 28 .	2,184,000	50	130,000	69	26·5	3·5	1
Oct. 9 .	2,000,000	50	81,450	70	25	4	1
,, 15 .	2,070,000	54	36,000	91	3	5	1
,, 20 .	2,300,000	60	6,200	75	5	20	0
Nov. 3 .	2,100,000	40	2,400	62	7	31	0

In a long series of differential counts only one nucleated red cell, a normoblast, was ever seen.

Histology. — *Small intestine.* — Epithelium largely shed. Villi short. Lieberkühn's glands well preserved. Eosinophiles were numerous in the mucosa and submucosa, but there was no crowding with leucocytes.

Vermiform appendix.—The interior was completely converted into fibrous tissue, in which were a few collections of myelocytes, eosinophiles, and lymphocytes.

Pancreas. — Healthy, except for some leucocyte infiltration round the larger veins.

Liver.—Leucocyte infiltration round the portal spaces, and to a less extent between the rows of liver cells. The liver cells were healthy, except for a great inequality in the size of their nuclei. There was a large quantity of iron pigment in the capillary endothelium, and in the liver cells towards the periphery of the lobules.

Spleen.—Malpighian bodies indistinct. There were some old infarcts. Many leucocytes in the pulp. There were several giant cells much degenerated. There was a considerable amount of iron pigment in large cells and lying free in the pulp.

Kidneys.—Kidney substance was healthy. Leucocyte infiltrations were noticed in the connective tissue under the capsule, along the vessels, and also scattered indiscriminately. Myelocytes, lymphocytes, polymorphs, and eosinophiles were present.

Suprarenal.—A few small groups of leucocytes were seen in the cortex.

Lymph glands.—A small inguinal gland showed normal structure. A large axillary gland showed lost structure, and in certain parts groups of myelocytes and eosinophiles, as well as lymphocytes. A mesenteric gland showed structure obliterated by crowded lymph paths. The blood vessels were congested. The capsule was invaded with myelocytes and eosinophiles. In a hæmolymph gland there was a great deal of pigment among the lymphocytes, not in the lymph paths. There were numerous large phagocytes in the lymph paths and under the capsule.

Cerebrum.—Healthy; no infiltrations.

Bone marrow.—Very little fat. The great majority of the cells were myelocytes. Eosinophiles were not very numerous. Red cells few, but a large percentage were normoblasts and megaloblasts. Giant cells were few and degenerated. There was some golden brown pigment here and there.

Case 6.—Male, æt. 42.

Histology.—*Stomach.*—There was some post-mortem shedding of the epithelium. Throughout the coats was an excess of polymorphs, lymphocytes, eosinophiles, and myelocytes. In the submucosa eosinophile myelocytes were numerous, and there were some basophiles with large dropsical-looking granules. There was no iron reaction.

Pancreas.—Healthy.

Liver.—There was slight leucocyte infiltration along the portal tracts. The leucocytes were of all varieties, including some very large lymphocytes. There were no giant cells and no phagocytes. At the periphery of the lobules the liver cells were rather atrophic, and contained a large amount of iron pigment.

Spleen.—Malpighian corpuscles were indefinite and small. Pulp was not very cellular, but all varieties of leucocytes were represented. Many normoblasts were seen at parts. Areas along the capsule and trabeculæ showed granular and rod-shaped masses of iron pigment. There were also areas

showing a network-like deposition of iron pigment in the pulp, possibly due to deposition along fibrin threads in old hæmorrhages.

Kidneys.—There was a fine infiltration of cells along the tubules, and some catarrh and congestion. There was no iron reaction.

Suprarenals.—There were a few scattered collections of leucocytes in the medulla.

Heart.—Healthy.

Lungs.—There were patches of catarrhal pneumonia. Only polymorphs occurred in the exudation.

Tonsils.—These were practically normal. There were no germ centres.

Lymph glands.—There was no definite gland structure. A few myelocytes and eosinophiles were seen among the lymphocytes. No iron. A mesenteric gland showed an old caseous nodule.

Bone marrow.—The fat had all gone. Except for an increase of eosinophiles, the leucocytes seemed in normal proportions. There were no phagocytes. Giant cells were much degenerated. There was no iron.

Case 7.—Man, æt. 60.

Histology.—*Tongue.*—Healthy.

Intestine.—Mucous membrane rather atrophic. Peyer's patches small, and composed entirely of lymphocytes.

Pancreas.—Healthy.

Liver.—There were a few myelocytes and other granular cells between the rows of liver cells, and a small number of polymorphs and myelocytes in some of the portal spaces. The liver cells were healthy. Here and there they contained iron pigment in small quantity.

Spleen.—Malpighian bodies indistinct. Some infiltration of pulp, with myelocytes and other granular cells. There were some granules of pigment which did not give the iron reaction.

Kidneys.—Practically healthy. Excess of leucocytes in the vessels.

Heart muscle.—Healthy.

Lungs.—Patches of catarrhal pneumonia and emphysema. Cells in exudation were polymorphs and catarrhal cells.

Medulla oblongata.—Increase of granular cells in the vessels.

Lymph glands.—There were very large phagocytes ingesting lymphocytes in all the lymph paths. There were a few basophiles and eosinophiles.

Bone marrow.—Fat had practically all disappeared. The most numerous cells were myelocytes and coarsely granular cells. Polymorphs were numerous. Lymphocytes were few. Nucleated red cells were numerous. No giant cells were seen. Phagocytes and pigmentary changes were not found.

III. Summary of Changes Found.

Alimentary canal.—In none of the six cases in which sections were examined were there accumulations of white cells sufficiently large to affect the structural arrangement. In only two cases was there any excess of cells, and these were mainly found in the submucosa.

Liver.—A prominent feature was the invasion of the portal spaces and hepatic capillaries with leucocytes. The packing of the portal spaces with blood cells was considerable in all the cases, with one exception; but the cellular excess in the capillaries varied very greatly in degree. In Case 1, while the portal spaces were not invaded, large

areas of liver tissue were rendered unrecognisable by the capillary engorgement. Myelocytes and other granular cells usually preponderated, but large lymphocytes were also present in large numbers in Cases 1, 2, 4, and 6. The liver cells were compressed, and atrophied wherever the capillary engorgement was great; but definite fatty degeneration was only found in one case. In only one case (1), that in which the capillary engorgement with white cells was most massive, were giant cells found. Normoblasts were numerous in the capillaries in three cases. Large phagocytes, ingesting red and white cells, or containing pigment, were found in the first four cases. Pigment in varying amount was present in the liver cells in all the cases, and gave the free iron reaction in six cases, but in none to so great an extent as in pernicious anæmia or acute lymphatic leukæmia.

Spleen.—In every case the Malpighian corpuscles were small. The pulp in six cases was packed with leucocytes as an outstanding feature. In Case 1 leucocytes were scanty, the pulp being congested; and in Case 3 it was fibrous. Nucleated red cells were present in fair numbers in five of the cases. Giant cells were numerous in two cases. Phagocytes ingesting red cells were found in three cases in varying numbers. Pigment was found in seven cases, and in six of these gave the free iron reaction.

Kidneys.—Catarrhal changes were present in four cases. In six cases there were white cell infiltrations, in some instances destroying the tubular structure. Giant cells were associated with these in one case. The epithelium of the tubules contained pigment in two cases. In one of these the iron reaction was present.

Lymphatic glands.—In four cases there was infiltration of the glands examined to such an extent as to obliterate their structure. Although a larger number of granular cells than normal was present, the bulk of the cellular excess was lymphocytic. An old caseous nodule was found in Case 6.

Hæmolymph glands.—In only two cases were these structures recognisable as such. Both showed lost structure and pigmentary changes. One case showed the free iron reaction.

Bone marrow was examined in six of the cases, having been lost in Case 3. In all it was definitely myelocytic, but eosinophiles were also a striking feature in Case 6. In two cases the proportion of cells other than myelocytes was insignificant. In the remaining four cases the bulk of cells other than myelocytes consisted of red cells. Normoblasts were present in all of the cases; megaloblasts in two of them. Phagocytes were seen in two cases. Pigment was present in two cases, and gave the iron reaction in one of them.

The main features of the cases are summarised in the following schedule:—

Number.	Sex.	Age.	Alimentary [anal] Cellular Excess.	Liver.							Spleen.						Kidneys.				Glands.		Hæmolymph Glands.		Marrow.						
				Infiltration.	Fatty.	Nonnoblasts.	Giant Cells.	Phagocytes.	Pigment.	Malpighian Corpuscles.	Pulp.	Nonnoblasts.	Giant Cells.	Phagocytes.	Pigment.	Catarrh.	Infiltration.	Giant Cells.	Pigment.	Structure lost.	Giant Cells.	Pigment.	Structure lost.	Phagocytes.	Pigment.	Type.	Second Type.	Megaloblasts.	Nonnoblasts.	Giant Cells.	Phagocytes.	Pigment.
1	M.	40	0	+ +	0	+	0	+ +	I	Small Red	Red	+	0	+	I	0	0	0	0	+	Small	0	+	I	Mye-locyte	Red	0	+	Few	+	I
2	M.	31	+	I	+	+	0	+	I	,,	Leu-cocytic	+	0	0	I	+	+	0	0	+	+	0	+	...	,,	Mye-locyte	Red	0	+	0	0	0
3	M.	40		I	0	0	0	+	I	,,	,,	...	+	...	I	+	+	+	+	0	+	0	+	...	,,	Red	Red	+	+	...	...	...
4	M.	37	0	+	0	+	0	+	+	,,	,,	+	0	+	I	0	+	0	0	0	...	0	...	...	...	,,	Mye-locyte	+	+	Few	+	0
5	M.	44	0	+	0		0	0	I	,,	,,	...	+	+	I	+	+	0	0	0 +	...	0	...	+	...	,,	Mye-locyte	+	+	,,	0	0
6	M.	42	+	I	0	0	0	0	I	,,	,,	+	0	+	I	+	+	0	0	+	...	0	...	...	...	Red	Myelocyte and Bo-sinophile	0	+	0	0	0
7	M.	60	0	+	0	+	...	0	...	,,	,,	+	0	0	0	0	+	...	...	0	+	...	...	...	...	,,	Mye-locyte	0	+	0	0	0

IV. Blood Counts.

The following table shows the result of enumeration and differential counts of the leucocytes in twenty-four cases made when the patient first came under observation :—

No.	Sex.	Age.		Polymorphs.	Large Lymphocytes.	Large Mononuclears.	Small Lymphocytes.	Eosinophiles.	Neutrophile Myelocytes.	Eosinophile Myelocytes.	Basophiles.	Per 300 Leucocytes.	
												Normoblasts.	Megaloblasts.
1	M.	43	...	37	3	7	0·3	5·6	34	4	9	0	1
2	F.	28	350,000	48	4·6	2.4	1·2	0·9	32	3·2	6	1	0
3	F.	21	412,000	24	3	6	1	8	53·7	3	1	0	0
4	M.	45	210,000	26	7	4	0	7	53	2	1	6	3
5	M.	50	...	58	0·5	1·5	0·5	0·5	36	2·5	0·5	3	1
6	F.	47	170,000	10	15	1	0·5	5	65	3	0·5	0	0
7	F.	39	470,000	61	0	0	0·5	0·5	37	0·5	0·5	22	10
8	F.	32	500,000	53	3·5	1.5	1	4	24	0·5	12·5	2	2
9	F.	37	400,000	64	1·5	1	1·5	6	15	4	7	5	0
10	F.	23	8,000	42	19	3	6	4	13	1	12	6	12
11	M.	33	116,562	50	4	0	1	1·5	41	2	0·5	7	7
12	F.	35	380,000	66	4	8	1·5	0·5	18	1	1	3	0
13	M.	40	451,875	18	2	2	1	2	68	1	7	0	0
14	M.	42	238,000	41	23	4	4	6	11	2	9	6	6
15	M.	31	320,000	66	4	3	1	1	20	5	0	2	4
16	M.	51	136,000	77	3	6	3	2	2	1	6	2	0
17	M.	50	640,000	37	3	3	1	1	47	8	0	6	3
18	M.	37	580,000	33	12	4	4	2	26	5	14	18	12
19	M.	29	450,000	60	3	2	4	3	24	2	2	9	9
20	M.	13	212,000	49	15	5	2	3	15	5	6	0	0
21	F.	9	130,000	32	15	7	3	5	22	3	13	6	33
22	M	60	...	64	2	1	0	5	27	1	0	6	18
23	F.	7	59,000	53	13	3	3	3	22	2	1	9	14
24	F.	42	420,000	48	10	3	1	3	16	4	15	0	0

As will be seen, the numbers of the total counts of leucocytes in the twenty-four cases cited are generally within the usual limits of from 200,000 to 500,000. The one case which has a really low count was in complete remission at the time. The striking point which comes out in the study of the percentages is their extreme variability in different cases, and, though this does not appear in the table, in the same case at different dates. Thus we find the following variations :—

Polymorphs	10	to 77	per cent.
Neutrophile myelocytes . .	2	,, 65	,,
Lymphocytes (all sorts) . .	3	,, 13	,,
Eosinophile polymorphs . .	0·5	,, 8	,,
,, myelocytes . .	0·5	,, 8	,,
Basophiles	0·5	,, 15	,,

This marked variability was pointed out by Ehrlich long ago, and, like him, we do not find that the alteration of percentages corresponds to any noticeable clinical differences or pathological variations. The difference in the individual case may be illustrated by taking the first one, in which, on four separate occasions, the percentages were as follows :—

Polymorphs	18	19	31	7
Myelocytes	68	44	46	58
Lymphocytes	2	30	18	34
Basophiles	7	7	1·6	6

The striking point that comes out in all the counts is the number of nucleated reds, and especially of megaloblasts, which were present. The normoblasts run from 0 to 22 per 300 leucocytes; the megaloblasts from 0 to 33. Both were absent from five cases on the first examination, and in one only were no nucleated reds ever found—a chronic relapsing case which has had several remissions. This patient is now very ill indeed, and very anæmic, but in spite of that fact there are no nucleated reds to be found in the films. We did not expect to find so many megaloblasts, and they were not by any means found mainly in late cases. A count of 12 per 300 whites was found in one case where the leucocytes were only 8000, and the case was symptomatically well with a decrease in the size of the spleen. Another count of 7 per 300 whites was found in a man doing full work, whose leukæmia was discovered accidentally. In cases which we followed out, however, they seemed to increase towards the end, and curiously enough in one, at least, where this was the case, they were not found in the marrow, all the nucleated reds there being normoblasts. The highest count of megaloblasts was in a case from the Sick Children's Hospital—an instance of the well-known fact that the anæmias of childhood are specially prone to assume the megaloblastic type. It seems to us that the presence of so many nucleated reds in the blood, especially when the anæmia is not very

marked, is a strong argument against the theory of an infectious origin for the disease, and in favour of that of a bone-marrow disturbance from other causes.

We thought that it would be of interest in view of the discussions which have recently taken place as to the place of origin of lymphocytes to find what the total number of these cells per c.c. of blood was. We are, of course, strongly of opinion that most, if not all, of the lymphocytes circulating in the blood are derived from the bone marrow, and in practically all the cases which we have cited the total number of lymphocytes was enormously increased. The numbers, indeed, were so great that one could not possibly imagine them coming from elsewhere than the marrow. If we take half a dozen cases at random the totals work out in this way—

Total counts—412,000, 400,000, 8,000, 116,500, 136,000, 451,800
Total Lymphocytes—41,200, 16,200, 2,240, 5,825, 14,820, 22,590
Lymphocyte percentage—10, 4, 28, 5, 12, 5.

V. Pathogenesis.

Löwit's view that leukæmia is due to a hæmamœba is now generally discredited, though he continues to publish observations. There is no other very definite theory as to causation. Ehrlich, we presume, adheres to his theory of mixed-cell leucocytosis. A fair number of experimental observations which have been undertaken with the view of throwing light on causation have been published within recent years. Schupfer (1905) injected leukæmic blood into rabbits, guinea-pigs, and bats. The rabbits showed a leucocytosis not leukæmic in type; the guinea-pigs died, probably because the injected blood was toxic or hæmolytic; the blood of the bats showed no change, though they are sensitive to organisms of the same type as the presumed hæmamœba. He also injected leukæmic blood into four patients suffering from carcinoma. The results should, of course, have been positive if the disease were due to an infection with organisms circulating in the blood. The patients lived varying times after the injections. The first showed a leucopenia; the second an ordinary leucocytosis as before the injection; the third a leucocytosis with an increase of lymphocytes, as before the injection; while the fourth received blood from an acute lymphatic leukæmia, and the pre-existing leucocytosis diminished. Löwit's bodies were never found in the blood of these patients. Masetti and Rubinato (1905), working along somewhat similar lines, injected leukæmic blood intravenously into rabbits in doses of from 10 to 15 drops, and placed portions of leukæmic spleen and marrow in the peritoneum. All the rabbits died after a few days, when the tissue was used; after one or two months, when blood had been injected. The authors state that a temporary leucocytosis was produced, generally running about 10,000, once up to 50,000. Mast

cells were increased, myelocytes appeared, and sometimes normoblasts, and there was a neoplastic hyperplasia of lymphatic tissue and marrow. Marrow from these animals carried on into others caused the same appearances. No organisms were found in the blood or organs of the human leukæmics, but in the organs of the rabbits staphylococci, bacilli, and diplococci were found. They regarded the lesions produced as being due to some toxic agency which escapes bacterial and microscopical investigation. It is curious, moreover, that leukæmia is not known to occur in these animals. Weil and Clerc (1905) find that it is common in the dog, horse, cattle, and pig; rarer in the cat; still rarer in the goat, sheep, and rat; unknown in the guinea-pig and rabbit. They found that intravenous injection of the blood of a leukæmic dog into another dog was fruitless. It was also unsuccessful when the blood was injected into the peritoneum, when the leukæmic tissues were transplanted under the skin, and when blood was injected into the veins of rabbits.

For very much the same reasons as those given in our recent paper on lymphatic leukæmia we regard myelocythæmia as a useless overgrowth of marrow cells, possibly due to the disturbance of the mechanism governing cell production—something akin to sarcomatosis. The origin of the conditions may differ, since a sarcoma always begins locally, while leukæmia is probably not a localised process in the same strict sense in the beginning, though possibly the great mobility of the tissue involved may account for the early diffusion of the process. As the elements involved are mainly granular cells in myelocythæmia, and as these are more highly differentiated than the lymphocytes, the disease is less malignant, and therefore in the great majority of cases runs a more chronic course than lymphatic leukæmia.

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EXPERIMENTAL AND CLINICAL OBSERVATIONS ON THE URINE AND BLOOD IN NEPHRITIS.[1]

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The following observations have been carried out:—

1. On two dogs and eight rabbits, in which experimental nephritis had been induced by the hypodermic injection of potassium chromate. In each of these a qualitative and a quantitative analysis of the urinary constituents was carried out, and observations were made on the blood.

2. Similar observations on the human subject were carried out, in one case of acute parenchymatous nephritis, one case of subacute parenchymatous nephritis, and one case of interstitial nephritis.

Methods.

In animal experiments the poisons commonly used, cantharides, corrosive sublimate, and oxalic acid, are difficult to regulate, and when the changes in the blood are to be noted the results are undesirable on account of the hæmolytic effect of these substances.

The agent found most suitable for prolonged administration was a salt of chromic acid; that used in these experiments was the potash salt.

Chromic salts were found suitable by Hall and Herxheimer.[2] They state, that apart from the toxic action on the organism the changes in the kidney, etc., are so regular that any direct effect, *i.e.* the effect on the tissues produced directly by the salt as opposed to the effect due to the nephritis, is minimal. The strength of the solution used in these experiments was 0·1 grm. of potassium chromate dissolved in 20 c.c. of water. The dose varied with the weight of the animal, the usual standard taken was 10 c.c. (0·05 grm. potassium chromate) for an animal of 1500 grms.

The first rabbit received 0·1 grm., but the action was so severe that the animal died in two days. In several rabbits the injection was repeated three times, 0·05 grm. of potassium chromate being given on each occasion. The animals were kept in a zinc cage, the floor of which inclined to a central drain. This drain was filled with glass wool through which the urine filtered free from fæces, etc., to a receptacle below.

[1] Received July 20, 1908.

[2] *Brit. Med. Journ.*, London, 1904, vol. i. p. 819.

The Diet during the whole Experiments.

I. Rabbits.—A constant green vegetable diet, in the form of cabbage, was given, as upon such the daily excretion is more abundant and regular than on the usual laboratory diet of meal and water.

II. Dogs.—The dogs were fed with unsalted oatmeal porridge and milk.

The following protocols give the results of two typical experiments in rabbits.

EXPERIMENTS IN RABBITS.

Experiment 5.

Rabbit (2230 grms.).—0·05 grm. of potassium chromate in 10 c.c. water was injected. On third day after injection the urine contained albumin 2·5 per mille, renal epithelium, and granular tube casts. For the next three days albumin was 0·5 per mille. Deposit—a few renal cells, and portions of tube casts. Weight, 2170.

Reinjected (0·75 grm. potassium chromate in 15 c.c. of water), and on the third and fourth day after that albumin equalled 2·5 per mille. Deposit, as before.

A third injection was given (0·1 grm. potassium chromate). Albumin in urine for day following rose to 3 per mille. Second day, 2 per mille. Deposit, renal epithelium with granular and hyaline tube casts.

The nephritis gradually subsided, and on the twelfth day the rabbit was found dead.

Changes in the Blood.

	Red Blood Corpuscles.	White Blood Corpuscles.	Polymorphonuclears.	Lymphocytes.	Basophiles.
Before injection . .	6,870,000	7,800	39	54	6
After injection, 4th day .	6,440,000	18,000	47	47	6
2nd ,, 1st ,, .	6,150,000	10,000	37	57	6
,, ,, 3rd ,, .	6,130,000	8,100	46	48	6
,, ,, 14th ,, .	6,500,000	7,800	44	50	6
3rd injection, 2nd ,, .	5,520,000	13,400	63	52	5
,, ,, 8th ,, .	6,280,000	7,200	50	44	6

Histological Changes in Organs.

Spleen.—Pulp markedly congested. Malpighian bodies were small. In the sinuses were large cells, both endothelium and leucocytes, engorged with slightly altered red blood corpuscles, or with a golden brown pigment derived from them. In addition to this there was a small quantity of a darker brown pigment in small granules, for the most part free and to a less extent intracellular. Some of the sinuses showed a marked prominence of the endothelial cells lining them.

Marrow.—Showed marked congestion and loss of normal arrangement of blood vessels. Increase of cellular elements encroaching upon the tissues, especially fat cells. Leucocytes seemed diminished in number, and more widely scattered among the erythrocytes than usual, a very few showed mitosis. Giant cells scanty and degenerated. Erythroblastic tissue not active.

Kidney.—Glomeruli showed slight fibrous thickening. Bowman's capsule

showed active proliferation. In some of the glomeruli there was accumulation up to the capillaries. In the convoluted tubules the epithelial cells were swollen and nuclei were not visible. Casts numerous. In the loop tubules active cell proliferation was seen.

Liver.—Throughout the liver there was a moderate quantity of a granular brown pigment; this was rather more in amount towards the periphery than the centre of the lobules. In addition to this, the endothelium lining the near capillaries was, in many cases, packed with a similar pigment. There was no evidence of congestion.

Experiment 6.

Rabbit (1845 grms.).—Potassium chromate, 0·05 grm. in 10 c.c. of water, was injected. On the following day 450 c.c. of urine was excreted, containing albumin, 1·5 per mille; few epithelial casts in the deposit.

The second day after injection the urine fell to 40 c.c.; albumin rose to 4 per mille.

On third day albumin amounted to 3 per mille; deposit similar. The albumin gradually disappeared from the urine, and after the fourteenth day only a trace could be found.

A second injection was given (0·05 grm. potassium chromate). Subsequent nephritis was slight. Albumin, 0·5 per mille for the next five days.

Third injection (0·05 grm. potassium chromate). No urine excreted on the following day, but on the second day the quantity rose to 240 c.c. There was a heavy precipitate of albumin.

Changes in the Blood.

	Red Blood Corpuscles.	White Blood Corpuscles.	Polymorphonuclears.	Lymphocytes.	Basophiles.
Before injection . .	7,530,000	10,000	53	45	2
After injection—					
1st injection, 1st day .	7,200,000	9,200	44	52	4
,, ,, 3rd ,, .	7,500,000	16,000	60	40	2
,, ,, 5th ,, .	8,220,000	10,000	45	45	10
,, ,, 8th ,, .	7,300,000	12,000	54	40	6
,, 12th ,, .	7,900,000	10,000	48	44	8
2nd injection, 2nd ,, .	6,000,000	10,500	. .	...	.
,, ,, 8th ,, .	6,600,000	13,000	. .	..	...

Histological Changes in Organs.

Spleen.—Congested. Malpighian bodies were small and comparatively inactive. Endothelial cells in sinuses were very prominent, and in the sinuses there were large cells which contained a dark brown pigment.

Marrow.—Congested. Fat cells shrunken and diminished: erythroblastic action was diminished, total number of red cells greatly reduced, and very few nucleated red blood corpuscles visible. There was marked evidence of red cells destruction. Scattered through the marrow, but tending to collect in areas, were large cells (as seen in the spleen) which had engulfed red blood corpuscles; these might be seen lying in the cells in all stages of disintegration. Leucoblastic tissue unusually active, the number of eosinophiles and pseudo-eosinophiles seen in all stages of mitosis being very great.

Kidneys.—Showed marked congestion of blood vessels and glomeruli,

in a few instances hæmorrhagic effusion between the glomeruli and capsule and into the surrounding tissue present. Epithelium of convoluted tubules was swollen, nuclei stained indistinctly ; lumen very much narrowed and filled with desquamated cells. Descending, ascending, and loop tubules of Henle showed desquamation of cells to a very much slighter degree. Collecting tubules practically unaffected. Early interstitial changes seen throughout the kidneys.

Liver.—Slightly congested. A few granules of pigment in the cells at periphery of lobules. In the hepatic capillaries, here and there throughout the liver, were granular leucocytes in all stages of mitosis.

Effect of Nephritis on Amount of Urine (Rabbits).

The results in this connection can, perhaps, be best compared by expressing the daily quantity of urine passed after injection, as a percentage of the urine before injection.

Experiment.	Days.					
	First.	Second.	Third.	Fourth.	Fifth.	Sixth.
1	0	85	...	...	...	...
2	82	182	...	...	...	...
3A	43	53	48	37	77	...
3B	53	40	48	63	76	...
3C	65	65	52	44	53	...
3D	58	19	40	46	...	...
4A	70	50	90	...	...	...
4B	50	0	101	41	45	...
4C	54	0	35	...	...	...
5A	56	21	44	64	120	...
5B	48	32	44	40	98	98
5C	40	84	56	101	...	...
6A	19	57	69	56	88	...
6B	23	101	150	76	85	...
6C	100	0	101	95	...	...
7A	117	103	91	63	100	...
7B	68	58	62	34	...	...
8A	90	90	91	95	...	...
8B	34	50	100	...	...	...
8C	95	0	0	..	...	...
	1165	1040	1212	855	743	98
Number of days observed	20	20	18	14	9	1
Averages	58	52	67	61	82	98

We find there is a marked fall in the amount of urine passed after the onset of the nephritis. This change is most marked during the first two days, when the urine fell to an average figure of 50 per cent. ; after this the amount gradually rises again, and by the seventh day the normal amount may be reached.

Protein per Diem expressed in Milligrammes (in Rabbit Experiments).

Experiment.	Days.					
	First.	Second.	Third.	Fourth.	Fifth.	Sixth.
1	0	700	...	...	...	...
2	170	250	...	...	...	...
3A	730	720	160	62	60	...
3B	60	50	60	70	60	...
3C	160	480	220	180	16	...
3D	60	400	320	190	...	...
4A	80	120	110	...	...	...
4B	120	0	730	220	100	...
4C	60	...	...	..	...	...
5A	0	0	275	80	160	...
5B	0	200	50	260	20	20
5C	300	420	70	130	..	...
6A	160	600	430	110	90	...
6B	25	120	160	80	70	...
6C	...	...	...	...	...	...
7A	40	180	160	110	170	...
7B	120	20	880	1200	...	..
8A	2	10	10	10	...	...
8B	0	50	100	...	...	...
8C	100	84	0	0	...	...
	2187	4560	3535	2662	746	...
	20	20	16	14	9	...
Average	109	228	220	190	82	20

Protein present in the Urine.

The table shows that the urine excreted the day after injection of potassium chromate contains albumin, and that the albumin is present in largest quantities during the second day. From the second day the albumin gradually subsides, till, by the seventh, the urine contains practically none.

When we compare the tables of daily excretion of urine and the total output of protein in the urine in twenty-four hours, it will be noticed

that as the protein rises the quantity of urine falls, and as the albumin disappears the urine tends to return to the normal level.

In three experiments (1, 2, and 10) the proteins were separated.

Experiment.	Total Protein.	Serum Albumin.	Serum Globulin.	Per Cent. of Total.	
				Serum Albumin.	Serum Globulin.
	Per Cent.	Per Cent.	Per Cent.		
1 . . .	0·35	0·27	0·07	75	25
2 . . .	0·078	0·05	0·028	66	34
	0·07	0·06	0·01	85	15
10A . .	1·29	0·9	0·38	64	36
	0·69	0·56	0·13	81	19
10B . .	0·17	0·12	0·05	73	27
	0·21	0·16	0·05	75	25
	0·14	0·10	0·04	71	29

The ratio of serum albumin to serum globulin is never constant. Serum albumin is present as a higher percentage of the total protein, and when the total protein is high the globulin is present in greater amount.

EXPERIMENTS IN DOGS.

Experiment 1.

Female dog.—Potassium chromate, 1·0 grm. in 20 c.c. of water was injected.

After injection—	
First day . . .	The urine was lost, as the dog suffered from diarrhœa.
Second ,, (560 c.c. of urine)	Slight amount of albumin ; deposit, nothing to note.
Third ,, (610 c.c.) . .	Traces of albumin ; deposit, few granular casts and epithelium.
Fourth ,, (555 c.c.) . .	Trace of albumin.
Reinjected	0·5 grm. potassium chromate.
Fifth day (730 c.c.) . .	Rise in amount of albumin.
Sixth ,, (710 c.c.) . .	Fairly heavy precipitate with nitric acid and on boiling ; deposit, granular tube casts.
Seventh ,, (370 c.c.) . .	Albumin and deposit similar.

The total nitrogen, chlorides, phosphates, and creatinin were estimated daily.

Excretion of Solids.

Total nitrogen.—The average total nitrogen for three days before induction of nephritis was 4·6 grms. per diem.

After the onset of nephritis—

Second day	3·7	grms.	per diem.
Third ,, ...	3·7	,,	,,
Fourth ,, ...	3·3	,,	,,
Fifth ,, ...	4·8	,,	,,
Sixth ,, ...	2·71	,,	,,
Seventh ,, ...	3·05	,,	,,

Average of the six days = 3·1 grms.

Chlorides.—The average output of chlorides for three days before induction of nephritis = 1·6 grm. NaCl per diem.

After onset of nephritis—

Second day	0·67	grm. per diem.
Third ,,	0·88	,, ,,
Fourth ,,	0·66	,, ,,
Fifth ,,	1·04	,, ,,
Sixth ,,	0·71	,, ,,
Seventh ,,	0·49	,, ,,

Average for the six days = 0·74 grm.

Phosphates.—Average for three days before induction of nephritis = 1·05 grm. P_2O_5.

After the onset—

Second day	0·87	grm. per diem.
Third ,,	1·22	,, ,,
Fourth ,,	0·79	,, ,,
Fifth ,,	1·43	,, ,,
Sixth ,,	1·10	,, ,,
Seventh ,,	0·84	,, ,,

Average for the six days = 1·04 grm.

Creatinin.—Average for three days before induction of nephritis = 0·435 grm.

After the onset—

Second day	0·392	grm. per diem.
Third ,,	0·449	,, ,,
Fourth ,,	0·320	,, ,,
Fifth ,,	0·492	,, ,,
Sixth ,,	0·383	,, ,,
Seventh ,,	0·297	,, ,,

Average for the six days = 0·383 grm.

The total nitrogen was reduced on the onset of nephritis from 4·6 grms. per diem to 3·7 grms. per diem, and still further when the albuminuria was more marked, namely, 2·71 grms. per diem.

The chlorides were excreted in less amount during the nephritis, falling from 1·6 grm. per diem to 0·67 grm. The amount rose as the albumin diminished, then there was another marked fall as the albumin increased again, namely, to 0·71 and 0·49 grm. per diem.

The phosphates and creatinin showed less variation. The creatinin follows the curve of the chlorides, but less closely. The phosphates show practically no reduction.

Summary.

	Nitrogen (Grms. per Diem).	Chlorides (Grms. per Diem).	Phosphates (Grms. per Diem).	Creatinin Grms. per Diem).
Average before nephritis . .	4·6	1·6	1·05	0·435
,, during the nephritis .	3·1	0·71	1·04	0·388

As regards the total nitrogen, the chlorides, the phosphates, and creatinin, we find the most distinct alteration in a reduction of total nitrogen and chlorides. To a less extent there is a diminution of the creatinin, and there is practically no alteration in the phosphates.

The total nitrogen is reduced by 25 per cent. and the chlorides over 50 per cent.

A considerable amount of work has been done clinically by French observers, chiefly by Widal and Javal (1903[1]). They state that in interstitial nephritis there is practically no reduction of chlorides, but in parenchymatous nephritis there is a marked retention of chlorides, leading to an increase of fluid in the tissues, this retention being proportioned to the albuminuria.

Labbé (1904[2]) contradicts this statement, and says there is no evidence of excess of chlorides in the blood.

In experiment 1 the very marked fall of the chlorides and the proportional rise in the albumin tend to confirm the contention of Widal and Javal.

Experiment 2.

In this experiment the proteins were separated.

Dog (14½ kilos).—1 grm. of potassium chromate injected in 20 c.c. of water.

	Protein Per Cent.	Serum Albumin.	Serum Globulin.
After injection—			
First day	1·29	0·9	0·38
Second ,,	0·69	0·56	0·13
Third ,,	No urine	...	...
Fourth ,,	0·122	...	...
Fifth ,,	0·08	...	...
Sixth ,,	0·04	...	...
Seventh ,,	0·02	...	...
Eighth ,,	0·018	...	...
Injection repeated			
First day	0·17	0·12	0·05
Second ,,	0·21	0·16	0·05
Third ,,	0·14	0·10	0·04

Changes in the Blood.

	Red Blood Corpuscles.	White Blood Corpuscles.	Polymorphonuclears.	Lymphocytes.	Eosinophiles.
Before injection	8,160,000	10,100	69	30	1
After injection—					
Second day	5,350,000	17,000	75	20	5
Fourth ,,	5,370,000	16,400	66	25	10
Eighth ,,	7,040,000	11,600	70	20	10
Second injection—					
Second day	6,470,000	23,200	75	20	5
Seventh ,,	7,870,000	16,800	63	26	9

Summary of Changes in Urine in Experimental Nephritis.

Amount.

1. The total urine per diem falls at the onset of nephritis, gradually rising again as the pathological condition subsides.

2. The amount of urine varies in inverse ratio with the actual amount of protein it contains.

Excretion of Solids.

1. Coinciding with the onset of nephritis there is a fall in the excretion of nitrogen, also marked retention of chlorides. To a less extent the creatinin output is diminished, and practically no alteration in the output of phosphates occurs.

Protein.

1. In view of its relationship to the output of total nitrogen and other constituents, the amount of protein present in the urine may be taken as an indication of the severity of the kidney lesion.

2. Serum albumin and serum globulin are always present, serum albumin always being in excess.

In the presence of a high albuminuria, serum globulin is in greater proportion than in a low albuminuria.

Changes in the Blood in Experimental Nephritis.

The following tables show the changes expressed as a percentage of the blood count before the induction of nephritis:—

Table I.—*Showing Changes in Percentage of Red and White Blood Corpuscles. Also Day of Experiment when Observation was made.*

	Red Blood Corpuscles.	White Blood Corpuscles.	Day of Experiment.
Experiment 2	93	125	Second.
,, 3	114	100	Seventh.
	96	96	Fourth.
,, 4	96	110	Fourth.
	96	108	Fourth.
	93	105	Tenth.
,, 5	93	230	Third.
	89	128	First.
	89	103	Third.
	94	100	Twelfth.
	80	171	Second.
	91	92	Eighth.
,, 6	95	87	First.
	100	150	Third.
	109	100	Fifth.
	96	118	Seventh.
	104	99	Twelfth.
	94	125	First.
	79	100	Fourth.
,, 7	79	125	Third.
	90	117	Fourth.
	95	114	Fourth.
,, 8	103	100	First.
	93	125	Third.
	82	142	Fourth.
	65	168	Second.
	66	162	Fourth.
,, 10	86	129	Ninth.
	79	229	First.
	96	166	Sixth.

TABLE II.—*Changes in Percentage of Red Blood Corpuscles arranged according to the Days of Experiment.*

First to Third Day.	Fourth to Sixth Day.	Seventh to Ninth Day.	Tenth to Twelfth Day.
93	96	114	93
93	96	91	94
89	96	96	104
89	109	89	
80	79		291
95	90	390	
100	95		3
94	82	4	
79	66		
103	96		
93			
65	905		
79			
	10		
1122			
13			
Average, 86	90·5	97·5	97

TABLE III.—*Changes in Percentage of White Blood Corpuscles arranged according to Days of Experiment.*

First to Third Day.	Fourth to Sixth Day.	Seventh to Ninth Day.	Tenth to Twelfth Day.
125	96	100	105
230	110	92	99
128	108	118	100
103	100	129	
171	100		304
87	117	439	
150	114		3
125	142	4	
125	162		
100	166		
125			
168	1215		
229			
	10		
1866			
13			
Average, 143	121	109	101

These tables show the following changes in the blood:—

1. The red cells during the first three days of the nephritis fall to an average figure of 86 per cent., gradually rising again during the next ten days to the normal percentage.

2. The white blood corpuscles show a marked increase during the

first three days to an average figure of 143 per cent., falling during the fourth to the sixth day to 121 per cent., and by the twelfth day they have practically reached the normal number. The increase in the leucocytes is accompanied by a rise in the number of polymorphs, as seen by the differential counts. In the dog experiment the resolution of the nephritis was accompanied by a marked increase in the eosinophiles.

These results accord with the common statement (Bezançon and Labbé [2]) that there is generally a fall in the number of the red cells, with an increase in the leucocytes on the onset of nephritis.

The leucocytosis of nephritis seems to be fairly constant even though associated with other pathological conditions. Gulland (1901 [3]) has pointed out that in several cases of pernicious anæmia the onset of nephritis was accompanied by a leucocytosis, while a pneumonia occurring in such cases does not usually produce any leucocytosis.

In scarlet fever Bowie (1902 [4]) has shown that the onset of nephritis is accompanied, or even preceded, by a distinct increase in the white cells.

Cabot (1904 [5]) says there is a slight diminution of the red cells in acute nephritis, but a leucocytosis is only present when there is some complication, such as uræmia or loss of blood by the kindney.

Clinical Observations on Nephritis.

Case 1.—*Acute Parenchymatous Nephritis.*

URINE.

	Daily Urine in c.c.	Specific Gravity.	Reaction.	Protein.
February 5 .	275 c.c.	1017	Acid.	7 per cent.
,, 6 .	1230 ,,	1016	,,	6·5 ,,
,, 7 .	1300 ,,	1015	,,	7 ,,
,, 8 .	1360 ,,	1013	,,	6 ,,

NITROGEN.

	Total Nitrogen in Grms. per Diem.	Urea Nitrogen in Grms. per Diem.	Ammonia in Grms. per Diem.	Creatinin in Grms. per Diem.
February 5 .	1·56	1·35	0·084	0·3
,, 6 .	7·65	5·8	0·270	1·0
,, 7 .	4·81	4·03	0·416	0·74
,, 8 .	4·0	3·06	0·340	0·94

Note.—The diet in cases 1 and 2 was similar—milk and milk foods. In case 3 the patient was on convalescent diet—fish, fowl, etc.

CASE 2.—*Subacute Parenchymatous Nephritis.*

URINE.

	Daily Urine in c.c.	Specific Gravity.	Reaction.	Protein.
February 5 .	900 c.c.	1022	Acid.	0·1 per cent.
,, 6 .	1440 ,,	1016	,,	0·05 ,,
,, 7 .	2520 ,,	1013	,,	0·05 ,,
,, 8 .	2350 ,,	1014	,,	0·05 ,,

NITROGEN.

	Total Nitrogen in Grms. per Diem.	Urea Nitrogen in Grms. per Diem.	Ammonia in Grms. per Diem.	Creatinin in Grms. per Diem.
February 5 .	10·26	8·73	0·45	0·9
,, 6 .	8·8	8·00	0·32	1·4
,, 7 .	10·5	8·10	0·2	1·5
,, 8 .	8·4	7·40	0·8	1·6

CASE 3.—*Chronic Interstitial Nephritis.*

URINE.

	Daily Urine in c.c.	Specific Gravity.	Reaction.	Protein.
November 15	1740 c.c.	1015	Acid.	0·018 per cent.
,, 16	1420 ,,	1020	,,	0·016 ,,
,, 17	1820 ,,	1018	,,	0·05 ,,
,, 18	1200 ,,	1022	,,	0·074 ,,

NITROGEN.

	Total Nitrogen in Grms. per Diem.	Urea Nitrogen in Grms. per Diem.	Ammonia in Grms. per Diem.	Creatinin in Grms. per Diem.
November 15	10·3	9·5	0·278	0·93
,, 16	15	12·6	0·36	1·4
,, 17	16·8	15·4	0·56	1·2
,, 18	13·4	12·4	0·48	1·62

SEPARATION OF PROTEINS.

	Total Protein.	Serum Albumin.	Per Cent.	Serum Globulin.	Per Cent.
November 15 .	0·018 per cent.	0·01	55	0·008	45
,, 16 .	0·016 ,,	0·01	63	0·006	37
,, 17 .	0·05 ,,	0·035	66	0·015	34
,, 18 .	0·074 ,,	0·05	66	0·024	34

The results of my experiments are not in accordance with the latter part of this observation.

In my experiment, No. 2, there was a great rise in the eosinophiles as the albuminuria passed off. This change has been recorded by Pieraccini (1901 [6]); he states that in the early acute stages the eosinophiles may be greatly reduced, but as the case resolves they reappear and may be in great excess. This has also been noted by Lesson (1900 [7]).

Comparison of Cases.

Total Nitrogen.

Case 1.	Case 2.	Case 3.
Grms. per Diem.	Grms. per Diem.	Grms. per Diem.
1·56	10·2	10·3
7·65	8·8	15
4·81	10	16·8
4	8·4	13·8
Aver. . 4·5	9·3	13·8

Taking the daily excretion, we find there is a great variation from day to day.

This is most noticeable in the case of acute parenchymatous nephritis, whereas in the case of subacute nephritis the daily amount shows comparatively slight alterations.

The amount excreted in all three cases is reduced 13·8, 9·3, and 4·5 grms. per diem.

The kidney lesions in the case of acute parenchymatous nephritis cause a marked reduction of the excretion of nitrogen, the amount rising as the pathological changes pass off. In the case of the chronic interstitial form the reduction is slight.

Nitrogen excreted in the Form of Ammonia, expressed as Percentage of the Total Nitrogen.

Case 1.	Case 2.	Case 3.
5·1 per cent.	4·2 per cent.	2·7 per cent.
3·5 ,,	4·8 ,,	2·7 ,,
8·1 ,,	2·0 ,,	3·5 ,,
8·4 ,,	2·0 ,,	3·5 ,,

The nitrogen excreted in the form of ammonia is remarkably high in the acute parenchymatous type (as high as 8·4 per cent.),

while in the subacute case the highest is 4·8 per cent., and in the insterstitial case only 3·5 per cent.

Creatinin.—Creatinin is excreted in a fairly steady amount in Case 2 and Case 3, and is only slightly altered in Case 1.

Summary of Results in three Clinical Cases.

1. *Total Nitrogen*

In the case of acute parenchymatous nephritis there was a marked reduction and irregular excretion, whereas in the subacute case the amount rises and is more regular. In the interstitial form the total nitrogen in not much below normal.

2. *Urea Nitrogen.*

The urea nitrogen follows the same course as the total nitrogen.

3. *Ammonia Nitrogen.*

In the case of acute parenchymatous nephritis the percentage of nitrogen excreted as ammonia is comparatively high.

4. *Creatinin.*

The creatinin excretion is only slightly interfered with in acute parenchymatous nephritis, and in the other two cases the excretion is fairly regular and high.

Summary of Chief Results.

1. In acute experimental nephritis, caused by potassium chromate, in rabbits, there is a diminution in the quantity of the urine excreted to the extent of nearly 50 per cent. on the first two days. After this the quantity gradually returns to normal.

2. The daily output of albumin is small on the first day of the induced nephritis. It rises to a maximum on the second day, after which it gradually disappears. The quantity of urine varies in inverse ratio with the output of albumin.

3. In three experiments the proteins were separated. Serum albumin and serum globulin were always found to be present. Serum albumin was always higher in the percentage of the total protein. In the case of a high albuminuria serum globulin was present in greater proportions than in a low one, but the ratio is not constant.

4. During experimental nephritis in a dog, the total nitrogen was reduced by 20 per cent., the chlorides by 50 per cent., while the creatinin was slightly reduced. The phosphates showed practically no alteration.

5. In three contrasted cases of nephritis in the human subject the results were as follow:—

		Acute Parenchymatous Nephritis.	Subacute Parenchymatous Nephritis.	Interstitial Nephritis, Chronic.
1.	Total nitrogen . .	Reduced, with great daily variations.	Reduced and practically constant.	Slightly reduced, with trivial variations.
2.	Ammonia per cent. of total nitrogen .	High, with daily variations.	Low.	Low.
3.	Creatinin	Slightly reduced.	Slightly reduced.	Practically normal.

The urea nitrogen follows the total nitrogen in each case.

6. In seven rabbits and a dog—(1) the red blood corpuscles showed an average fall of 14 per cent. on the onset of the nephritis, and gradually rose again to normal; (2) the white blood corpuscles rose to the extent of 43 per cent., gradually returning to the normal as the albumin disappeared. The curve of the white blood corpuscles followed the curve of the protein in the urine. The differential counts showed an increase in the polymorphs, and these showed a return to normal *pari passu* as the nephritis subsided.

7. The histological changes in the organs, induced by potassium chromate, in the rabbit were acute nephritis, chiefly affecting the tubules and, to a slightly less extent, the glomeruli; a remarkable congestion and phagocytosis of red blood corpuscles in the spleen; slight pigmentary changes in the liver, and a marked leucoblastic hyperplasia in the bone marrow, followed by degeneration. These changes were remarkably constant, and the description in one case might equally apply to any of the others.

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THE CLINICAL VALUE OF THE PANCREATIC REACTION IN THE URINE, BASED ON OVER 250 ANALYSES.

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In recent years the clinical researches of Mayo Robson and other observers have directed attention to the comparative frequency of serious organic disease of the pancreas and to the various clinical tests which assist in its recognition. More particularly, attention has been directed to the value of the pancreatic reaction in the urine, a test devised by Cammidge, which has proved in Mayo Robson's experience of great value in diagnosis. This test, first described by Cammidge in 1904, has been elaborated and improved, and in 1908 is again advanced by its originator as a valuable diagnostic method, after an experience of 800 analyses.

My interest in this subject was aroused in the course of the autumn of last year through the admission to Sir Thomas Fraser's wards, then under my care, of a case of chronic pancreatitis, in which this diagnosis had previously been established by operation. The object of the present paper is to present a brief summary of results I have obtained in a series of over 250 analyses from 120 consecutive cases. The test employed was the "improved method" as described in Mayo Robson's and Cammidge's recently published work on *Diseases of the Pancreas*. I have empirically divided the positive results obtained into three groups:

(*a*) *Pronounced.*—Those in which the end reaction showed a pronounced deposit of the crystals of characteristic appearance and solubility, the deposit ranging from about ¼ in. to ½ in. in an ordinary test tube.

(*b*) *Marked.*—Those in which the deposit was much less in amount, but still considerable.

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To the Psoric Acari belong two groups, namely, Sarcoptidæ and Demodecidæ.

[1] Received November 16, 1910.

(*c*) *Slight.*—Those in which there was either a slight deposit on naked-eye examination or in which there was no naked-eye deposit, the presence of crystals only being determined on microscopic examination.

NOTE.—At the outset of my observations I experienced some difficulty with the test and failed to get any reaction. This failure may have been due to faulty chemicals employed. At a later date Dr. Cammidge kindly demonstrated to me the end reaction, and subsequently I have experienced no special difficulty with the test. The present series of analyses was carried out by a trained assistant in the laboratory of the Royal College of Physicians, the end reaction being determined by myself, a microscopic examination being made in every instance.

PRONOUNCED REACTION.

Clinical Diagnosis.	Remarks.
1. Chronic pancreatitis ..	Pronounced reaction during acute attacks, on two occasions negative in interval.
2. Acute pancreatitis ..	Operation: Incision and drainage; cholecystenterostomy (Mr. Caird). Reaction gradually became less marked and was negative when patient was discharged cured.
3. Acute suppurative appendicitis and peritonitis	Died. Pancreas showed the naked eye and microscopic appearances of acute pancreatitis.
4. Malaria (aestivo-autumnal)	Jaundice with pronounced epigastric tenderness. Reaction became negative coincidently with recovery from jaundice and epigastric tenderness.
5. Pneumonia	Died. Pronounced reaction three days before death; reaction negative on day of death. Pronounced arterial sclerosis. No *post-mortem* examination.
6. Chronic bronchitis, arterio - sclerosis, and myocarditis	Pronounced reaction on three occasions. Man, aged 48. Incapacitated from work.
7. Alimentary glycosuria and constipation	Marked diminution in the degree of reaction coincidently with use of restricted diet and aperients.
8. Duodenal ulcer, chronic pancreatitis	Pronounced reaction associated with glycosuria. Extensive malignant disease was revealed at the operation.
9. Gall stones in common bile duct	Reaction negative on two occasions in December, 1907; slight positive reaction on day before operation (January 10th), reaction became very marked and patient died on fourteenth day. Pancreas showed the naked eye and microscopic appearances of recent inflammation.
10. Exploratory laparotomy	Palpable tumour in region of pancreas. Pronounced pancreatic reaction with trace of glycosuria; at the operation Professor Chiene found marked chronic pancreatitis.
11. Pregnancy, seventh month	Pronounced reaction associated with glycosuria (patient not seen by the author)
12. Mitral stenosis (pronounced)	Reaction negative on admission. A pronounced pancreatic reaction developed coincidentally with the occurrence of jaundice, epigastric tenderness, and general increase in severity of the symptoms. Died. At the *post-mortem* examination there was intense catarrh of duodenum, with muco-purulent blocking of the ampulla of Vater and recent inflammatory changes in the pancreas.

MODERATE REACTION.

Clinical Diagnosis.	Remarks.
13. Chronic pancreatitis...	Probably a primary infective inflammation unassociated with gall stones. Symptoms of ten years' duration.
14. Uraemia	Died. At the *post-mortem* examination the terminal portion of the common bile duct was markedly bile stained throughout its course.
15. Colitis	Associated with pale stools of some years' duration.
16. Uraemia	Recovery. Reaction negative on discharge from hospital.
17. Gall stones in common bile duct	Reaction negative in December, positive in January, before operation; increased in degree after operation, and finally disappeared on recovery.
18. Acute gout	The administration of 1 grain of calomel at night for some days was followed by a copious discharge of apparently unaltered bile.
19. Tuberculous intestine Phthisis	Died.
20. Chronic pancreatitis secondary to duodenal ulcer	Reaction negative in December, positive in January, and associated with glycosuria, followed by melaena and exacerbation of symptoms.
21. Constipation	Symptoms of six years' duration, ill-defined tumour in region of hepatic flexure. Reaction became negative after eight days' close attention to the bowels (eight enemata of warm oil, followed by soap and water, with, internally, castor oil, etc.).
22. Chronic nephritis, alcoholism	Patient not personally seen.
23. Cerebral haemorrhage Aphasia	Pronounced arterial disease. History of two previous cerebral haemorrhages.
24. Exophthalmic goitre.	Died in February, 1908. In December, 1907, it was observed that there was a notable, though temporary, improvement in patient's condition coincidently with the administration of pancreatic emulsion.
25. Exophthalmic goitre.	Died. Pancreas showed chronic interstitial inflammation, with areas of small-celled infiltration.
26. Gastric ulcer	One examination made a few days before discharge from medical ward.
27. Malignant disease of stomach	Four analyses made after operation, reaction positive with distinct glycosuria.
28 Chronic pancreatitis..	Woman, aged 63. Abdominal pains eight months' duration Marked pancreatic reaction with considerable glycosuria. May be secondary to malignant disease.

SLIGHT REACTION.

Other 18 cases gave a slight reaction. In 5 of these cases there was no trace of deposit visible to the unaided eye, the reaction being determined by microscopic examination only. This group includes cases of broncho-pneumonia, appendicitis, myelogenous leukaemia, malignant disease of the liver, cardiac disease, chronic nephritis, chronic bronchitis, arterial sclerosis, chronic intestinal obstruction due to new growth, simple catarrhal jaundice, and a fatal case of lymphosarcoma. In the last mentioned the reaction was negative on repeated examination after

To the Psoric Acari belong two groups, namely, Sarcoptidæ and Demodecidæ.

[1] Received November 16, 1910.

the patient's admission to hospital, but the reaction was positive on two occasions during the last week of life.

Negative Results.

A negative reaction, in many instances after repeated examination, was given in 75 cases. They included cases of

jaundice due to calculus in the common bile duct (the calculi being passed per anum), jaundice due to malignant disease, gastric ulcer, duodenal ulcer, indigestion and constipation, malignant disease of stomach, abdominal aneurysm, malaria (aestivo-autumnal), cancer of the uterus, rectal carcinoma, various diseases of heart, lungs, kidneys, and bladder, and a fatal case of anaemia and thrombosis.

The last mentioned is of special interest. During the last few days of life the symptoms, fever, collapse, abdominal pains, etc., closely simulated acute intra-abdominal inflammation, and the reaction of the urine was negative on two examinations. At the *post-mortem* examination the pancreas appeared perfectly healthy, and on microscopic examination of different parts of the gland an unusually good picture of a perfectly normal gland was found.

Comments.

It is of interest to refer in greater detail to some of the data which appear to warrant the conclusion that this reaction in the urine has a special relation to pancreatic disease. These data are furnished both from the clinical and pathological standpoint. For clinical facts reference may be made to the following cases:

Case 2, acute pancreatitis, successfully operated on by Mr. Caird, in which ten analyses were made, the reaction becoming less pronounced, and finally being negative on the patient's discharge from hospital cured.

Cases 4, 8, 12, and 21, recently under treatment in Sir Thomas Fraser's wards, are also noteworthy. Case 4 is specially interesting since there was a useful control. Two seamen were admitted to the wards on the same day suffering from well-marked malaria of malignant type. One of these (Case 4) was distinctly jaundiced; there was marked tenderness on pressure in the epigastric region, and the urine gave a pronounced pancreatic reaction, which became negative coincidently with the disappearance of the jaundice and the tenderness on pressure in the epigastric region. The other sailor was not jaundiced, the epigastric region was free from tenderness on deep pressure, and the pancreatic reaction was invariably negative (four analyses).

Case 8 is instructive, since the pancreatitis was associated with, and apparently a sequel of, duodenal ulcer, and was further complicated by glycosuria, doubtless of pancreatic origin. As already indicated, it was found at the operation by Mr. Caird that the pancreatitis was secondary to malignant disease.

Case 12 illustrates another point, namely, the development of the reaction in consequence of the congestion and catarrh, probably of infective origin, that may be present in advanced cardiac disease. In this patient the reaction was negative on admission, later the reaction was positive though slight, a pronounced reaction developing coincidently with the occurrence of jaundice, epigastric tenderness, and a general increase

in the severity of the symptoms. At the *post-mortem* examination the duodenum showed intense catarrh, the ampulla of Vater was distended with muco-pus and the pancreas showed considerable swelling and detachment of the secreting cells with much oedema of the interstitial tissue.

Case 21 appears to show that the pancreatic reaction in the urine may result from a disturbance of the function of the pancreas from pressure of a faecal mass.

In this patient there was an ill-defined tumour in the region of the hepatic flexure, the subjective symptoms being abdominal pains and general weakness of some years' duration. The pancreatic reaction in the urine became negative coincidently with the relief from the constipation and general improvement in the patient's condition.

Cases 9 and 10 have special interest from the surgical point of view.

In Case 9, a female patient, deeply jaundiced, the reaction was negative on two occasions in December, a slight positive reaction was obtained on January 10th, the day before operation, the operation revealing several calculi impacted low down in the common bile duct with extensive adhesions around. The reaction became very pronounced, and the patient died on the fourteenth day, the pancreas showing the naked-eye and microscopic appearances of recent inflammation. The history of this case suggests that the chances of the patient's recovery were lessened from the fact that in the few weeks prior to operation the inflammation round the bile duct had extended and involved the pancreas.

Case 10.—This patient had a well-defined tumour in the epigastric region, with a pronounced pancreatic reaction in the urine, and slight glycosuria. At the operation Professor Chiene found that the tumour was an enlarged and indurated pancreas.

Before leaving these cases it is of interest to note that I have recorded in the above series no fewer than 5 cases of chronic pancreatic disease, which exhibited a marked pancreatic reaction, with associated glycosuria. Some details of two of these are given above (Cases 8 and 10). The others are Case 27, in which the *post-mortem* findings confirmed the diagnosis of chronic pancreatic disease; Case 26, a patient under the care of Professor Greenfield, regarded as pancreatitis secondary to duodenal ulcer; and Case 20, in Sir Thomas Fraser's wards, in which the pancreatitis may be secondary to malignant disease of the gland. In this connexion it is of interest to note that in 65 consecutive cases of chronic pancreatitis operated on by Mayo Robson, glycosuria was detected in 4 cases only.

From the pathological point of view, the facts so far obtained in the *post-mortem* room confirm the conclusion arrived at from the clinical data as to the relationship of the pancreatic reaction to disease of the pancreas. In one patient, a case of chlorosis with widespread thrombosis, dying with a high temperature and symptoms resembling acute intra-abdominal inflammation, the reaction was negative on repeated examination, and the naked-eye and microscopic appearances of the pancreas were in all respects normal; whereas in 7 cases which gave a marked positive reaction, definite inflammatory or degenerative changes were present in the gland.

To the Psoric Acari belong two groups, namely, Sarcoptidæ and Demodecidæ.

[1] Received November 16, 1910.

General Conclusions.

My results confirm the conclusion arrived at by Mayo Robson and Cammidge, that there is a definite and important relationship between the pancreatic reaction in the urine and disease of the pancreas. My results differ from those of Cammidge in so far that I have recorded a higher percentage of positive results in cases similar to those that may have been included in that author's list of control cases. Cammidge found in a group of cases described as miscellaneous—other than those having a direct surgical interest in relation to disease of bile ducts and pancreas—that 4 only out of 92 gave a positive reaction. The higher percentage of positive results recorded in my series may possibly be due to the fact that I made use of many serious medical cases of a nature commonly associated with advanced arterial disease, and chronic catarrhal conditions in the intestinal tract, conditions that are frequently attended with degenerative or inflammatory changes in the pancreas and other glands. My results lead me to divide the cases in which the pancreatic reaction was present in my series into the following groups:

1. A group in which there was definite clinical or pathological evidence of serious organic disease of the pancreas—for example, acute and chronic pancreatitis, usually associated with disease of the bile ducts.

2. A group in which the reaction in the urine is associated with pronounced arterial sclerosis, a condition usually accompanied by more or less sclerosis in different glands.

3. A group in which the reaction is dependent on congestion and catarrhal conditions of the gland ducts and substance, with associated toxaemia — for example, advanced heart disease, appendicitis, pneumonia, malaria, and the like.

This division is, it is hardly necessary to add, somewhat arbitrary, as most cases are in all probability of mixed origin. If these facts are kept in view, and this urinary reaction is carefully studied along with the other clinical features of a case, I have no hesitation in saying that this new test will prove of great value both to physicians and surgeons in the diagnosis and treatment of pancreatic disease.

I have much pleasure in expressing my indebtedness to Sir Thomas Fraser and other senior colleagues at the Royal Infirmary for the facilities afforded me in this investigation.

THE USE OF OIL IN ABDOMINAL SURGERY[1]

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DURING the past twenty years the use of oil in the peritoneal cavity has on several occasions been recommended by various writers, who have brought forward different indications for its employment. Thus in the numerous records of experiments carried out with a view to finding some agent which would prevent the formation of post-operative adhesions, sterile oil is almost invariably mentioned as one of the agents tried. The intraperitoneal use of oil has also been advocated for cases of peritonitis with the object of delaying the absorption of bacteria from the peritoneal cavity. The use of this agent has not, however, received much favor among the profession generally, probably because surgeons are loath to introduce into the peritoneal cavity any foreign substance concerning the fate of which they are uncertain. Accordingly one must first ascertain whether or not there be any danger associated with the introduction of oil into the abdomen before advocating its use in abdominal surgery. The dangers which suggest themselves are oil embolism, peritoneal irritation by this foreign substance, and sepsis. With regard to oil embolism it may be at once stated that no observer has ever recorded any evidence that

[1] From the Laboratory of the Royal College of Physicians, Edinburgh.

cattle, dogs, etc., so frequently. These Non-psoric Acari are only temporary parasites, and do not, as a rule, produce very severe symptoms.

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it does occur, at any rate in a degree sufficient to cause harm. Personally in some thirty experiments one has always carefully examined post-mortem for any evidence of this complication, but one has never detected any lesion indicating its presence.

Concerning the question of irritation or infection of the peritoneum by the oil different opinions have been expressed. Thus, Stern (1) found considerable inflammatory reaction in the peritoneal cavity of rabbits after the introduction of presumably sterile olive oil. Blake (2) on the other hand saw no evidence of peritoneal irritation after the introduction of large quantities of sterile oil into the peritoneal cavity in cats. The difficulty here consists in the fact that in order to sterilize any ordinary oil, such as olive oil, one must expose it to a temperature which causes the separation of some free fatty acid, which is undoubtedly irritative. In the first series of experiments the writer employed the purest sterile olive oil, but found that it caused considerable peritoneal irritation. After unsuccessfully trying to obtain a purely animal oil, he, at the suggestion of Mr. Alexander, chemist to the Edinburgh Royal Infirmary, tried *sterile vaseline oil*, and after extensive use of this agent, is quite satisfied that it is the best for the purpose. It is the oily liquid which comes to the surface in the preparation of vaseline, it is really a liquid paraffin, is absolutely neutral, and remains unchanged after prolonged exposure to steam under high pressure. One can confidently recommend the use of this agent in the peri-

toneal cavity in suitable cases as being absolutely free from danger.

THE USE OF STERILE OIL IN THE TREATMENT OF PERITONEAL ADHESIONS

The formation of adhesions after abdominal operations is a subject which has received the attention of surgeons ever since the advent of the era of abdominal surgery. The question of the prevention of such adhesions naturally has been studied most carefully, and very numerous methods have been devised with this purpose. At the present day our knowledge of the subject stands somewhat as follows: After an aseptic abdominal operation carried out without undue or rough handling of the viscera adhesions are unlikely to occur. Simple abrasion of the peritoneal surface of the viscera without infection may, but seldom does, lead to permanent adhesions. Infection of the peritoneal cavity may clear up and leave no permanent adhesions. Rough handling and abrasion of peritoneal surfaces in infected cases generally lead to the formation of adhesions, which may be permanent. In several experiments on cats the writer has found that serious abrasions of considerable peritoneal surfaces may in some cases be produced without the subsequent formation of any lasting adhesions, provided no infection of the peritoneum is permitted. Even where infection was lacking, however, some degree of adhesion of the denuded surfaces was the rule.

In the human subject idiosyncrasy would

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seem to play a large part in determining the formation of adhesions, and an operation which in one person would cause very little peritoneal disturbance, in another gives rise to extensive adhesions, the treatment of which is notoriously unsatisfactory.

After an ordinary aseptic abdominal operation, however, there is little justification for the routine practice of introducing some foreign substance such as oil into the peritoneal cavity with a view to preventing adhesions. More practical interest centers in the question regarding what is to be done for the patient who presents himself for treatment with symptoms referable to peritoneal adhesions. Laparotomy and separation of the adhesions with the burying of raw surfaces as far as that is possible, gives as a rule only temporary relief. The introduction of foreign substances between the rawed surfaces has frequently been tried. Stern experimented with sterile vaseline, olive oil, and collodion, but found none of them to be of real value. Duschinsky (3) tried the insertion of pieces of gold beaters' skin, and Lauenstein (4) experimented with silk protective, but neither proved to be of practical value. Vogel (5) obtained some success with a mucilage of gum arabic. Busch and Bibergeil (6) in an elaborate research tested olive oil, gum arabic, paraffin, lanoline, gelatine, coragen, agar, and fibrolysin, but found none of them of any great value. They used oil in only one experiment, however, so that with regard to it their conclusions do not carry much weight. More recently Blake has recorded in this jour-

nal his experience in the use of oil in the abdomen, and is of the opinion that it does tend to prevent the formation of adhesions. Gellhorn experimented with sterilized lanoline and found that this prevented adhesions to some extent.

Numerous writers recommend pouring a saline solution into the abdomen at the conclusion of an operation as a preventive measure against adhesions, but the rapidity of absorption of this fluid makes its use of questionable value.

Relative to this part of the subject the writer has carried out six experiments on cats and rabbits. The procedure was as follows: The animal's abdomen was opened with aseptic precaution, the surface of the small intestine over about sixteen inches of its length, was scrubbed with dry gauze and scraped with a knife till bleeding points appeared. Six weeks or two months later the abdomens of the twelve animals so treated were reopened and in all adhesions were present, though they were never very extensive. These adhesions were broken down leaving raw surfaces. In six of the animals oil was then smeared over the rawed surfaces and the abdomen closed. In the control animals the adhesions were broken down but no oil used. The animals were opened again four weeks later, and it was found that in those in which oil had been used, the adhesions were very much fewer and less dense than in the controls. At the end of a month all trace of oil had disappeared from the peritoneal cavity. One has found fifteen days to be the longest time for oil to

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remain in macroscopic quantity in the peritoneal cavity in aseptic cases.

One concluded, therefore, that sterile oil may be advantageously used for mopping over denuded surfaces in operations for peritoneal adhesions.

THE USE OF OIL IN CASES OF ACUTE PERITONITIS TO DELAY ABSORPTION OF BACTERIA

An enormous capacity for absorption has long been recognized as a striking characteristic of the peritoneum. One need not recapitulate the results of the work done in this connection, but need only mention that as the result of researches by Wegner (7), Starling and Tubby (8), Meltzer (9), Mendel (10), Klapp (11), Orlow (12), Heidenhain (13), Durham (14), McCallum (15), Buxton and Torrey (16), and Peiser (17), we have now fairly accurate knowledge as to the process of absorption from the normal peritoneal cavity. We know that fluids and soluble substances are absorbed in great part by the blood stream, whilst solid particles, including bacteria and such things as oil globules are absorbed by the lymphatic. We know, moreover, that the lymphatic absorption is most active on the under surface of the diaphragm and in the omentum and is very sluggish in the pelvis. Regarding the influence of peritonitis on the absorption from the peritoneal cavity, widely different results have been recorded by different observers. Schnitzler and Ewald (18) found that the absorption of potassium iodide from the peritoneal cavity was delayed in peritonitis.

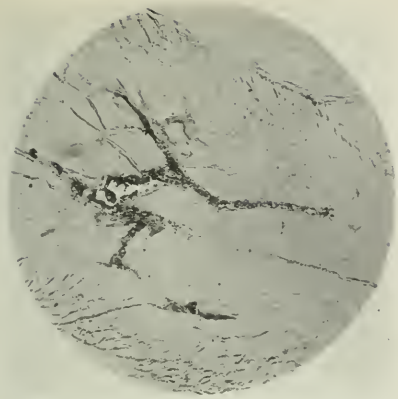

Fig. 1. Section of rabbit's diaphragm seven weeks after injecting oil and staphylococci into its peritoneal cavity. Note oil in phrenic lymphatics. (Sudan III.)

Clairmont and Haberer (19) found that absorption was accelerated by peritonitis, except in the very late stages of the malady, when it was slightly retarded. Glimm (20) in an elaborate research found that absorption of lactose was invariably accelerated during peritonitis, and his results appear to be of greatest value. We may take it, therefore, that the absorption of fluids and of soluble substances is increased during peritonitis.

Peiser (21) investigated the absorption of bacteria from the peritoneal cavity into the blood stream during peritonitis. He found, during

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Fig. 2. Acute obstruction due to recent adhesions. Note great distention of stomach and jejunum and the collapsed ileum lying to the right of stomach. A, position of the adhesion causing obstruction.

the first few hours of a peritonitis, great numbers of bacteria in the blood stream, but during the course of the peritonitis as the number of bacteria increased in the peritoneal effusion, the number in the blood stream diminished, owing, he thought, to the lymphatics of the diaphragm becoming mechanically plugged by bacteria. The fibrous exudate coating the

diaphragm must, however, also play a large part in retarding the absorption of bacteria.

The question now arises as to *which is the more favorable for the patient suffering from peritonitis, rapid or slow absorption?* Many writers, most prominent among whom was Lennander (22), considered that absorption of bacteria into the blood stream, by stimulating the production of antibodies, was a protective and beneficial occurrence. More recent work, however, has shown that this is not the case, and that on the contrary the more localized the infection can be kept the greater the chance for the patient. Peiser found that a certain dose of B. Coli. could be injected with impunity into the peritoneal cavity of a rabbit. The same dose could be injected into the blood stream of a rabbit without producing death. If, however, one half of this dose was injected into the peritoneal cavity, and an hour later the other half was injected into the blood stream, the rabbit died within a few hours with all the signs of an acute peritonitis. It would appear therefore, that as Peiser says, "Recovery from peritonitis depends on the fight between the bacteria and the body defenses being confined to the peritoneal cavity."

DOES THE INTRAPERITONEAL ADMINISTRATION OF OIL RETARD BACTERIAL ABSORPTION IN PERITONITIS?

Glimm investigated this point and as the result of his experiments concluded that the administration of oil greatly retarded bacterial absorption and favored recovery from

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peritonitis. He, however, injected the oil some hours before inducing the peritonitis, and as Buxton and Torrey (23) have shown, Glimm's good results were mainly due to the reactive changes set up in the peritoneum by the preliminary introduction of the oil. The writer carried out a series of twelve experiments to test the value of oil as a life saving agent when it is given at the same time as or shortly after the peritonitis is induced, this being obviously the question of most practical import.

The results of these experiments are given in tabular form below. These experiments were carried out on rabbits. The bacterium employed was staplylococcus aureus, the latter because, as the writer has elsewhere shown,[1] it induces in rabbits a purulent plastic type of peritonitis closely resembling that found in the human subject.

The purest sterile clive oil was employed, and except where otherwise noted, it was injected immediately before the introduction of the bacteria. The record of these experiments is given in tabular form (see Table 1).

From these experiments it is clear that the presence of oil in the peritoneal cavity of a rabbit with a generalized peritonitis *with no adhesions*, does not have any noteworthy beneficial action as regards the saving of life.

At the same time the results in experiments 2 and 8, seemed to indicate that the oil may have some influence in averting early death from rapid absorption, and in localizing the infection to the peritoneal cavity, and might

[1] Jour. of Path. and Bact., October, 1900.

TABLE I

	Animal Used	Oil Injected	Bacteria Injected	Result
Exp. 1.	Rabbit 1.	2.5 cc. oil.	200 million St. Aur.	Recovery.
	Rabbit 2, control.	None.	200 million St. Aur.	Death 3 weeks later.
Exp. 2.	Rabbit 1.	3.5 cc. oil.	4,000 million St. Aur.	Death 7 weeks later.
	Rabbit 2, control.	None.	4,000 million St. Aur.	Death 10 hours after.
Exp. 3.	Rabbit 1.	3 cc. oil.	4,000 million St. Aur.	Death 14 hours after.
	Rabbit 2, control.	None.	4,000 million St. Aur.	Death 15 hours after.
Exp. 4.	Rabbit 1.	2 cc. oil.	3,000 million St. Aur.	Death 48 hours later.
	Rabbit 2, control.	None.	3,000 million St. Aur.	Death 484 hours later.
Exp. 5.	Rabbit 1.	4 cc. oil.	2,500 million St. Aur.	Recovery.
	Rabbit 2, control.	None.	2,500 million St. Aur.	Recovery.
Exp. 6.	Rabbit 1.	4 cc. oil.	3,500 million St. Aur.	Death 23 hours after.
	Rabbit 2, control.	None.	3,500 million St. Aur.	Death 224 hours after.
Exp. 7.	Rabbit 1.	5 cc. oil.	3,000 million St. Aur.	Death 54 hours after.
	Rabbit 2, control.	None.	3,000 million St. Aur.	Death 33 hours after.
Exp. 8.	Rabbit 1.	4 cc. oil.	4,500 million St. Aur.	Recovery but emaciated
	Rabbit 2, control.	None.	4,500 million St. Aur.	Death 13 hours after.
Exp. 9.	Rabbit 1.	4.5 cc. oil.	1,000 million St. Aur.	Death 9 hours later.
	Rabbit 2, control.	None.	1,000 million St. Aur.	Death 13 hours later.
Exp. 10.	Rabbit 1.	4 cc. oil.	1,500 million St. Aur.	Death 17 hours later.
	Rabbit 2, control.	None.	1,500 million St. Aur.	Death 164 hours later.
Exp. 11.	Rabbit 1.	5 cc. oil 3 hrs. after.	1,000 million St. Aur.	Death 2 weeks later.
	Rabbit 2, control.	None.	1,000 million St. Aur.	Recovery.
Exp. 12.	Rabbit 1.	3 cc. oil 2¼ hrs. after.	2,500 million St. Aur.	Death within 15 hrs.
	Rabbit 2, control.	None.	2,500 million St. Aur.	Death within 15 hrs.

possibly be used with advantage in e. g., fulminating cases of appendicitis.

The next question requiring solution is, *Does the presence of oil in the peritoneal effusion interfere in any way with the cellular reaction in that effusion, and does it in non-fatal cases delay the process of recovery?* By making films at intervals of a few hours from the peritoneal effusion in two rabbits with peritonitis, to one of which oil had been given, one was able to

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note the cellular changes. It was found that the cellular reaction was not interfered with by the oil and that the return to normal was only slightly delayed. There were one or two exceptions however (Exper. 2, 8, and 11) where the animals got over the initial danger but then gradually emaciated and in which at the end of two or more weeks on opening the abdomen, one found a cake of "cream cheese like" material which on examination was found to consist of fibrin, degenerated leucocytes, oil globules and bacteria. One cannot therefore agree with Glimm that the presence of oil never impedes but rather hastens the return to normal in the peritoneal cavity. That the oil may, and generally does, remain for long periods in the phrenic lymphatics was made evident by microscopic sections of the diaphragms of rabbits at various intervals after the injection of the oil. In one case oil globules were still present in the phrenic lymphatics seven weeks (see Fig. 1) in another ten and a half weeks after the intraperitoneal injection.

Cultures were made from the heart blood of all the fatal cases in the above experiments, and it was interesting to find that whereas a growth of staphylococcus was obtained on five occasions from the blood of the control animals which got no oil, on only one occasion was a similar growth got from the blood of the animals which had received an injection of oil. As the death rate among the latter animals was almost as high as among the former ones, this is but another proof that the fatal issue in cases of peritonitis is due to toxæmia and not to sep-

ticæmia, and it would appear to upset somewhat the rationale of this method of treatment.

THE USE OF OIL TO PREVENT OBSTRUCTION FROM RECENT PLASTIC ADHESIONS IN CASES OF ACUTE PERITONITIS

In many fatal cases of peritonitis, more especially those due to appendicitis, a common post-mortem finding is great distention of the small intestine, particularly of the jejunum. This is generally regarded as being the result of a toxic paresis of the bowel and that the toxic factor plays a large part in the etiology of such cases is undoubted. In many cases, however, the writer is convinced that a definite obstructive cause is present, and that this cause is the binding together by recent plastic adhesions of some of the coils of small intestine and usually of the coils of the ileum to each other, to the cæcum, or to some abraded area of the parietal peritoneum. At the upper limit of the adhesion some difficulty is offered to onward peristalsis, distention of the immediately proximal loop occurs and a kink forms.

One has had the opportunity during the past year to observe the course of five such cases. In all of these an operation was performed for peritonitis following appendicitis, and in each case the patient died some days later with vomiting and great abdominal distention. At the post-mortem great distention of the jejunum and upper part of the ileum was found, whilst the lower coils of ileum were collapsed and empty and between these two segments, some coils of ileum were found, bound by recent

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adhesions to each other, to the cæcum or to the pelvic wall. In such cases were the distention of a paralytic nature it would affect the entire small intestine and not only the upper part, leaving the lower part collapsed. Moreover if an enterostomy be made, in a distended loop in such a case, sufficiently early, it is not uncommon to see active peristalsis in the distended bowel, suggesting a distal obstruction and not a paresis or paralysis of the entire gut.

That a recent plastic adhesion is sufficient to cause definite intestinal obstruction one has succeeded in proving experimentally, and though the peristaltic power of the normal intestine would probably overcome such an obstruction, that of a bowel weakened by toxæmia is frequently inadequate. A minimum of handling of inflamed bowel at operations for peritonitis is now a generally recognized maxim, but when such handling must be practiced, as it sometimes must, the mopping of such handled surfaces with sterile oil in order to prevent subsequent adhesion, is a procedure which I believe is more than justified by the results of the experiments described below.

The two factors which one wished to introduce into these experiments were, firstly, abrasion of the peritoneum, and secondly, bacterial infection, the latter being of a mild nature and not of itself sufficient to endanger the animal's life. The procedure was as follows: Under ether anæsthesia the abdomen of a rabbit was opened, its appendix was amputated, and the stump invaginated. The cæcum and some inches of the ileum were scrubbed

with a piece of dry, sterile gauze to produce an abrasion of the peritoneal coat. One or two loopfuls of an emulsion of staphylococcus aureus (from one-fiftieth to one-twentieth of the lethal intraperitoneal dose) were then introduced into the peritoneal cavity and the abdomen was closed. The same procedure was carried out on a second rabbit with this addition, that before closing the abdomen some sterile vaseline oil was poured over the intestines.

EXPERIMENT I

Rabbit 1. Ether anæsthesia, abdomen opened, appendix amputated, cæcum and 12 inches of ileum scrubbed with dry sterile gauze. One-fiftieth of lethal dose of staphylococcus aureus suspended in saline injected into the abdomen, wound closed. The following day the animal was very dull and would not eat and it was found dead on the morning of the second day following operation.

Post-mortem. Great abdominal distention. On opening the abdomen enormously distended coils of jejunum presented. The stomach greatly distended. Cæcum and lower part of ileum collapsed and empty. On tracing the distended bowel downwards one found that it had adhered to the cæcum at one point. Here it had kinked on itself and led to complete intestinal obstruction. The adhesion was quite recent and broke down with the slightest traction. There was little free fluid in the abdomen and death was obviously due to obstruction and not to infective peritonitis.

Rabbit 2. Ether anæsthesia. Abdomen opened, appendix amputated. Cæcum and twelve inches of ileum scrubbed with dry gauze. One-fiftieth part of a lethal dose of staphylococcus aureus suspended in saline injected into peritoneal cavity. *Four cc. of sterile vaseline oil poured over the intestinol coils*, abdomen closed. The animal appeared well on the following day and made a perfect recovery. A week later under ether anæsthesia the abdomen was reopened. Free

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oil was still visible forming a thin film over the intestines and there were no adhesions, abdomen closed. Six weeks later the abdomen was opened a third time. No oil could now be seen and there were a few adhesions between the ileum and the cæcum.

EXPERIMENT 2

Rabbit 1. Ether anæsthesia, abdomen opened, appendix amputated, stump invaginated. Cæcum scrubbed with dry gauze till peritoneal coat roughened. Sixteen inches of the ileum similarly treated. One-twentieth part of a lethal intraperitoneal dose of staphylococcus aureus suspended in saline injected over the intestines; abdomen closed. The rabbit was very dull and would not eat on the following day and it died 44 hours after operation with a greatly distended abdomen.

Post-mortem. Great tumidity of the abdomen. On opening the peritoneal cavity a distended stomach and greatly distended coils of jejunum presented. Tracing the distended bowel downwards one came to a point where the small intestine had adhered to the cæcum. Here a kink was found to have occurred, several loops of intestine below this had also adhered to the cæcum, and the lower part of the ileum was collapsed and empty. The large intestine was more or less collapsed. There was practically no free fluid in the abdomen and but a mild degree of peritoneal inflammation. Death was obviously due to obstruction. (See Fig. 2.)

Rabbit 2. Ether anæsthesia; abdomen opened; appendix amputated, stump invaginated. The cæcum and sixteen inches of small intestine scrubbed with dry gauze and then one-twentieth part of the lethal dose of staphylococcus aureus in saline injected over the intestine. *Before closing the abdomen 5 cc. of sterile vaseline oil was poured over the intestines.*

The rabbit never appeared ill and made a prompt and complete recovery. Eighteen days later its abdomen was reopened. There were numerous adhesions of recent date. (From the ease with which they were broken down probably the adhesions were of not more than five or six days' duration.)

No trace of oil in the abdominal cavity. Three

other similar experiments were carried out. In one of these both rabbits made prompt recoveries. In the other two, the rabbits which got no oil were ill for two days, but recovered, whilst those which got oil never showed any signs of abdominal trouble.

Another case of death from obstruction due to a recent adhesion occurred in a rabbit, 4 days after infection of the peritoneum, without the mechanical abrasion of the peritoneum practiced in the experiments mentioned above. From these results two facts are quite clear: Firstly, that recent plastic adhesions (even of 36 hours' duration) are capable of causing complete intestinal obstruction, and secondly, that the use of sterile oil applied over abraded peritoneal surfaces in cases of abdominal infections is of great value in preventing obstruction from recent adhesions.

There is yet another question which still awaits an answer, and that is, whether when definite obstructive symptoms appear after an operation for, say acute appendicitis, it would be advisable to freely open the abdomen, break down the adhesions and cover the separated surfaces with some oily substance to prevent further adhesion. Whether the separation of adherent surfaces, by exposing abraded areas and thus hastening the absorption of toxins, would do more harm than the obstruction was previously causing, is a question which experience alone will decide. In any case mere opening and draining the distended gut, though always doing good, seldom avails to save the patient's life in this desperate class of case.

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THE USE OF OIL TO FAVOR DRAINAGE AND INTESTINAL PERISTALSIS IN CASES OF GENERALIZED PERITONITIS

When one examines the abdomen of a patient who has died, say two days after an operation for generalized peritonitis, one realizes how the property which the peritoneum possesses of forming plastic adhesions and which is so valuable in localizing infections, may when the infection becomes generalized, militate against the patient's chances of recovery. One finds in such a case that whereas at the operation, two days before, the intestines were floating in purulent exudate, at the post-mortem the coils of intestine are glued together and between them are everywhere pockets of pus. When the pus was evacuated at the operation the inflamed peritoneal surfaces came together and adhered, and the pelvic or other drains which were left in only drained a small area in their immediate vicinity. This gluing together of the intestinal coils must moreover have considerably interfered with the peristalsis of the bowel wall, weakened as it was by toxæmia. In such cases I believe we have the clearest indication of all for the use of oil in the peritoneal cavity. If at the operation, after the pus is evacuated or the abdomen washed out, oil be poured over the inflamed intestines, adhesions will be prevented, for a few days at least, the purulent exudate will find its way down to the pelvic drain and so escape, and the general lubrication will favor intestinal peristalsis.

Through the kindness of Professor Caird one

has had the opportunity of practicing this method of treatment in two cases of absolutely generalized peritonitis. In the one case in which the peritonitis was due to a perforation in a partially gangrenous cæcum which had herniated through the foramen of Winslow, and in which, at the operation, considerable exposure and handling of the intestines was unavoidable, two ounces of sterile vaseline oil was poured into the abdomen at the conclusion of the operation. The patient died 36 hours later and at post-mortem the intestines were found non-adherent and covered with a thin film of oil.

In the second case where an appendix abscess had ruptured into the general peritoneal cavity and where pus and fibrin were found covering the liver and under surface of the diaphragm the same procedure was carried out at the operation. The patient recovered and it was noteworthy that she passed flatus a few hours after operation, and had none of the symptoms of intestinal paresis which one usually sees in such cases.

CONCLUSIONS

From the observations recorded above one feels justified in advocating the introduction of vaseline oil into the peritoneal cavity in the following conditions:

1. In operations for the relief of old-standing adhesions.

2. In operations for localized or diffuse peritonitis where handling of the viscera is unavoidable.

cattle, dogs, etc., so frequently. These Non-psoric Acari are only temporary parasites, and do not, as a rule, produce very severe symptoms.

To the Psoric Acari belong two groups, namely, Sarcoptidæ and Demodecidæ.

[1] Received November 16, 1910.

3. In operations for generalized peritonitis to favor subsequent drainage and intestinal peristalsis.

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AN INVESTIGATION INTO SCABIES IN LABORATORY ANIMALS.[1]

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During the last three years, whilst working in this laboratory, my attention has been drawn to the prevalence of scabies amongst the animals commonly used there. As it is important that such animals should be in perfect health before being used for experimental work, it seems to me that a short description of the appearances of these diseases, the methods of diagnosis, the course which they usually run, and the measures to be taken to prevent their spread, may be of use to those engaged in animal research work.

Before proceeding to the description of the various varieties of scabies, it may be well to mention the exact zoological position which the parasites found in this disease occupy in the animal kingdom.

The various kinds of "mange" found in animals are all due to members of the order of Acarina. The Acarina form a special order in the class Arachnida. All acari (mites) have non-articulated bodies, the head, thorax, and abdomen being all one mass. They have a chitinous integument with spines and hairs, and their limbs are terminated by suckers or hairs. The sexes are separate, the females being larger and more numerous than the males. They all undergo a succession of changes as they develop. They are either oviparous or ovo-viviparous. From the egg they hatch out in from four to seven days as larvæ, which have as a rule only three pairs of legs (hexapod larvæ). After further development they acquire a fourth pair of legs, and reach the stage of nymphæ (or pupæ). Then the genital organs develop, and the animal becomes a pubescent male or female (or imago) fourteen to seventeen days after hatching. Lastly, after impregnation, the female becomes an ovigerous female. Friedberger and Fröhner (1908 [5]) state that the female dies in from three to five weeks after laying her eggs, and the male lives about six weeks. The same authors also quote Gerlach as stating that about 1½ million sheep acari spring from a single female in ninety days.

Acariasis in the Mammalia is divided into Non-psoric and Psoric Acariasis. To the former belong several groups, including the Ticks, which attack horses, cattle, dogs, etc., so frequently. These Non-psoric Acari are only temporary parasites, and do not, as a rule, produce very severe symptoms.

To the Psoric Acari belong two groups, namely, Sarcoptidæ and Demodecidæ.

[1] Received November 16, 1910.

Both these forms are more or less serious for the host which is attacked. The Sarcoptidæ live on or in the epidermis itself, the Demodecidæ practically exclusively in the sebaceous glands of the skin. With the first of these two groups this paper deals.

Psoric Sarcoptidæ.

These are divided into three distinct genera—(1) *Sarcoptes*, (2) *Psoroptes*, (3) *Chorioptes*.

The *Sarcoptes* (σαρξ, flesh ; κοπτειν, to cut) or burrowing mites are small, have rounded bodies and very short legs, and the hind-legs do not project beyond the edge of the body. The female lives in the horny layer of the skin, forming a burrow in which she deposits her eggs. The male lives on the surface of the skin, clinging to scales, etc.

The *Psoroptes* (ψώρα, from ψὼ, I rub, and πτήσσειν, I conceal) or sucking mites (also called *Dermatodectes* (Gerlach) or *Dermatocoptes* (Fürstenberg)), and the *Chorioptes* or scale-eating mites (also called *Symbiotes* (Gerlach) or *Dermatophagi* (Fürstenberg)) are much larger in size, and live on the surface of the skin and in its superficial layers, usually chiefly attacking the insides of the ears only.

Friedberger and Fröhner (1908 [5]) state that the *Sarcoptes* feed on the cells of the rete Malpighii of the skin ; the *Psoroptes* suck blood serum and lymph from the skin, clinging the while to scales and hairs ; whilst the *Chorioptes* feed on the younger epidermic scales which they gnaw away from the surface of the skin.

In the domesticated animals, *e.g.* horse, sheep, goat, dog, etc., the disease has been already very thoroughly studied. In this paper the forms of disease found in the rabbit, rat, mouse, cat, ferret, and fox will be described.

Rabbit Scabies.

Rabbits suffer from two forms of scabies due respectively to a *Sarcoptes* and a *Psoroptes*.[1]

The Sarcoptic Mange begins usually about the face as a scaliness around the eyes and on the nose (Plate XXXIV. Fig. 1). If the disease is left alone it spreads from these areas till the whole face is covered with a thick scaly eruption. The scales are whitish-yellow, and are mixed up with yellowish crusts and hairs. The disease soon spreads up to the bases of the ears on their outer aspect, and also in a circular manner round the neck. It never affects the insides of the ears. In the average case it goes no further than the localities mentioned, but in severe cases it spreads to the fore and hind-legs, into the groins and around the genitals. At first only scaling and crusting result, but later there is some loss of hair, but never to any very great extent. The itching seems to vary. The animals scratch themselves a good deal, but not so much as one would expect considering the number of acari present. If scales, etc., from any of the affected areas be removed and examined in liquor potassæ, sarcoptes are found in all stages of development mixed up with scales, crusts, and hairs. There is no difficulty in finding the acari, provided one scrapes off the scales,

[1] Friedberger and Fröhner also state that rabbits may be affected with *Chorioptes*, but I have not observed this.

etc., right down to the deeper part of the horny layer of the skin, as the acari are most numerous next the skin itself. These acari have the characters of all sarcoptes (Plate XXXIV. Fig. 3), being rounded in shape, with four pairs of legs, the anterior legs all having suckers, and the posterior legs in the females having long hairs at the end, whilst in the male, the inner pair has suckers, and the outer, hairs. The female is larger than the male, averaging 0·215 mm. long by 0·177 mm. broad, whilst the male measures 0·156 mm. long by 0·124 mm. broad. In the scales are also seen very numerous ova, young acari in the larval and pubescent stages, and fæces.

In examining scales, etc., for acari the best results are obtained by putting the material on a slide with a drop of liquor potassæ, covering with a cover glass and heating gently. The liquor potassæ clears up the scales in a few minutes; the acari become a light yellow colour, and stand out in contrast to the scales, etc. Sometimes it is difficult to get the acari separated from hairs, crusts, etc., and if so, the untreated material should be spread out in a Petri's dish and warmed gently, when the acari begin moving about and can be picked up on the point of a needle and mounted separately. If permanent specimens are desired, the liquor potassæ should be poured off, and a drop or two of water dropped on at one end of the slide and allowed to flow over the specimen. This is repeated several times so as to wash off all the caustic potash. Then the slide is warmed and a drop of glycerin jelly (gelatin and glycerin, part. aeq. with a crystal of thymol), which has been melted in a hot-water bath, put on and covered with a warm cover glass. When cool the glycerin jelly solidifies. Such specimens keep indefinitely, and are much better than those mounted in Farrant's solution or pure glycerin. One can also examine the scales, etc., in glycerin instead of in liquor potassæ, but they take longer to clear up, are more mixed up with air bubbles, and as the acari do not take on the yellow colour produced by the liquor potassæ, they are, therefore, not so easily seen. The glycerin like the liquor potassæ can be removed, and the specimen permanently mounted in glycerin jelly. One cannot mount acari in Canada balsam, as they are difficult to dehydrate completely. In this investigation the method described first (namely, liquor potassæ and then glycerin jelly) was found to give the most satisfactory results.

The skin from such cases was also examined microscopically. In examining the following forms of scabies the majority of specimens were put through paraffin, and a few through celloidin. The acari are well seen when stained by most stains, but the hæmatoxylin and van Gieson method gave the best results, the acari staining more darkly by this method than by any other.

In Sarcoptic Scabies of the Rabbit the acari are seen embedded deeply in the horny layer of the skin (Plate XXXIV. Figs. 2 and 5). The Malpighian layer is practically unaltered. Superficial to the acari there is a thick mass of scales and crusts, which is permeated all through by spaces,—the tunnels made by the acari as they move about. In these tunnels are débris, fæces, and ova. There is a slight small-celled infiltration of the corium. There is neither ulceration of the surface, nor formation of vesicles in the epithelial layers.

Once a rabbit is infected with sarcoptic scabies the disease spreads fairly rapidly, a well-marked eruption being present after three or four weeks. The condition is very serious if left alone, as the animal

becomes emaciated and dies from the irritation produced. When the disease is extensive around the mouth, it leads to cracking of the skin, with the formation of deep fissures, which are very painful when the animal attempts to eat, and that I think has a good deal to do with the emaciation which results. The animal sits with eyes half closed and takes very little notice of anything.

The disease spreads most easily from one rabbit to another by direct contact. If an animal suffering from the disease be placed beside healthy ones the disease is soon communicated. I have repeatedly removed, from a diseased rabbit, living sarcoptes and ova in all stages of development in considerable numbers, and placed them on the skin of healthy rabbits on the areas which are most usually affected, but have never succeeded in transmitting the disease by that means. The failure was not due to any insusceptibility on the part of the animal, for in one case where a rabbit had failed to show any signs of the disease after repeated attempts at artificial transmission, I placed it in a cage beside a diseased animal, and in a short time it showed marked signs of the disease. The disease may also be spread by infected cages in which diseased animals have been kept. But I placed a healthy rabbit in a cage out of which a diseased one had been taken, and although the cage was not cleaned out at all, the healthy rabbit did not develop scabies. Many animals which are kept by themselves in cages develop the disease, but I cannot say definitely whether they get it from the cages being infected or by transmission of acari or their ova from one animal to another by attendants. These methods are unlikely, for, as just stated, it is difficult to infect animals with small quantities of the acari and without prolonged contact of the infecting agent. A much more likely reason for the disease appearing in such animals is that they are infected whilst they are running about with the other stock rabbits which are all kept together ; when first put in a cage by themselves they look quite healthy but have a few acari on them, which soon increase in number, although it is only after a week or two that the condition becomes evident. As the cages here are frequently cleaned thoroughly, these are not likely to be the source of infection. Another possible method of spread is by the baskets in which the laboratory workers carry the rabbits to and from their cages for examination. If such a basket is infected it may readily spread the disease. The question of the transmissibility of the disease from one species of laboratory animal to another and to man is important. Neumann (1905 [1]) quotes Zurn as stating that rabbit scabies is transmissible to man, and says that it causes a trifling disturbance which lasts a few days only. The fact that none of the attendants or workers in the laboratory, although they are constantly handling infected animals, have ever shown any signs of irritation on the hands or elsewhere, points to the difficulty of transmitting the disease to man. From a comparison (see Table)

of the sizes of the sarcoptes of the different animals, the cat sarcopt is the one which most nearly approaches that of the rabbit in size. No attempts were made to infect cats from rabbits or *vice versa*, as the two animals could not be kept together, and as the mere placing of a few acari from one rabbit on to another rabbit did not produce the disease, still less likely is it that it would succeed between the rabbit and cat.

The sarcoptic scabies of the rabbit can, however, be readily transmitted to the guinea-pig. A guinea-pig, whose skin was quite healthy, was placed on 14th November 1909 beside a rabbit suffering from a sarcoptic and psoroptic scabies. A fortnight later the guinea-pig was scaly more or less all over the body and on the face, especially around the eyes. In the scales very numerous sarcoptes were to be seen. On 8th December 1909 another healthy guinea-pig was placed beside the same rabbit; it also in ten days showed marked scaly rings around the eyes (Plate XXXIX. Fig. 25), and a great deal of crusting and scaliness on the body, especially on the sides (Plate XL. Fig. 29). Amongst the scales, etc., sarcoptes were easily found. There is no doubt therefore that sarcoptic scabies of the rabbit is readily transmitted to the guinea-pig.

An attempt was also made to infect rats with sarcoptes from the rabbit, but although acari, etc., were placed on four rats, none of the animals ever showed any signs of scabies. Two rats were also placed each in the same cage beside a rabbit suffering from sarcoptic scabies. In neither case did the rat show any signs of the disease even after several weeks. This is not to be wondered at, as the sarcopt of the rat is slightly larger than that of the rabbit. That, together with the failure to transmit the disease, points to the rabbit and rat sarcopt being different species of acari.

In addition to a *Sarcoptes minor* in the rabbit, Friedberger and Fröhner (1908[5]) say that the tame rabbit may be infected with a *S. scabiei*, and that that form of the disease can be communicated to guinea-pigs and ferrets. I have examined many rabbits with sarcoptic scabies, but all had an acarus of the same small size belonging to the variety of *S. minor*.

The treatment of sarcoptic scabies in rabbits is very unsatisfactory. Neumann recommends washing with soft soap and then applying Helmerich's ointment (sulph. sublimat., 100 parts; pot. carb., 100 parts; lard, 800 parts). I have used ung. sulphuris (B.P.) but without success. Ointments are too thick and lie too much on the surface of the hairs and crusts. An ointment, consisting of a mixture of equal parts of soft paraffin and vaseline with a few drops of lysol, was also tried. The parts where the ointment was thoroughly applied became smooth, and a good deal of the hair fell out, but the disease soon spread again, especially from the eyes, for the animal closes the eyes firmly and prevents the application getting properly at the acari

in that region. The best measures to adopt to get rid of the disease are destruction of all diseased animals, disinfection of the cages, and a careful inspection of all new animals so as to prevent its reintroduction from the outside. These remarks apply to all animals used in the laboratory. The parts to be specially examined are around the eyes, about the ears and the genital region.

Psoroptic Scabies of the Rabbit (*Psoroptic otacariasis*).—Psoroptic scabies in the rabbit primarily affects only the insides of the ears. It shows itself as a crusting inside the ears at the bottom of the concha. After a time the whole inside of the ear becomes filled up with a great mass (Plate XXXV. Figs. 6 and 7), which is whitish-yellow in colour, dry, and arranged in thick flakes like pastry. This mass may be as much as three-quarters of an inch thick. Between the layers of scales and crusts are masses of fæces and a few dead acari. If all these scales are removed the surface of the skin is found to be red, moist, and oozing, and lying on it are thousands of acari. There is an offensive odour and the ears are somewhat tender to touch. These acari are about three times the size of sarcoptes, and are easily visible to the naked eye. They can be scraped off the inside of the ear in bunches, and are seen usually arranged in pairs, the male and female together (Plates XXXVI. and XXXVII. Figs. 11 and 12). The psoroptes are easily distinguished microscopically from the sarcoptes. Their large size and their relatively long hind-legs, which project well beyond the body of the animal, are characteristic (Plate XXXVII. Figs. 13 and 14). Their bodies are squarish, whilst the sarcoptes are rounder and their heads are more pointed. The suckers on the limbs have long, thin, three-jointed pedicles, whereas in the sarcoptes and chorioptes the pedicles of the suckers have no joints at all. The females have two long bristles on the hind-legs, and the males have two long outer hind-legs with suckers and two short inner ones with bristles. In addition the male has copulatory suckers. Mixed up with the adult acari are numerous ova and larvæ. These psoroptes do not form definite burrows in the skin. They lie on the skin, and by their irritation cause a moist exuding surface. The animal scratches the ears and shakes the head frequently. Often the psoroptes remain localised in the ears, and Neumann says that the disease never affects any other part of the body. But whilst it may be the rule to find psoroptes only inside the ears, I have seen in three rabbits, in addition to the eruption inside the ears, an extensive eruption on the face, neck, and limbs. In these cases only psoroptes were found. Zürn also records a similar spread of the disease. When it spreads to the body it produces a very great deal of scaling and crusting, and very soon loss of hair on the affected regions (Plates XXXVI. and XXXVII. Figs. 10 and 15). Masses of psoroptes may be literally scraped off the surface of the skin. The animal rapidly emaciates, the hair falls off the legs but not off the

feet, the eyes become closed and surrounded with crusts, the face fissured and cracked, and, if left alone, the animal rapidly dies. The irritation produced by the psoroptes is much more severe than that produced by the sarcoptes.

If a healthy rabbit is placed beside one with psoroptic scabies the disease is easily transmitted, but although, as in sarcoptic scabies I repeatedly placed acari and their ova from a diseased ear into that of a healthy rabbit, I never succeeded in transmitting the disease. The animals always shook the acari out or removed them with their hind feet. In order to prevent this, after placing acari inside the ears of two rabbits, the ears were covered with strapping, but with negative result. Possibly the absence of oxygen prevented their living. Psoroptes from rabbits were also placed inside the ears of four mice but with negative results. Müller and Zürn and others have reported finding psoroptes in the middle ear and mastoid cells of such rabbits, and have observed vertigo and symptoms of meningitis. Unfortunately the middle and inner ears of these rabbits were not examined.

On examining microscopically the skin from the ears of rabbits with psoroptic scabies, the acari are found lying on the inner surface of the ear, and superficial to them are several layers of crusts (Plate XXXIV. Fig. 4) consisting of masses of leucocytes and coagulated serum, blood, and epithelial cells. In between the layers of crusts are faeces, the acari themselves being found chiefly amongst the crusts near the skin itself. The surface of the epithelium (Plate XXXVII. Fig. 16) is abraded here and there and covered with a thick exudate. The epithelium is slightly hypertrophied, and the whole corium, subcutaneous tissue, and slightly also the superficial layers of the muscle, are infiltrated with a dense mass of small round cells. There are no giant cells. This infiltration varied considerably in amount in different cases, being in some cases much less than one would have expected.

Successful treatment of psoroptic scabies in rabbits is not easy, as the ears are tender and the animals resent their being touched. The same methods as for sarcoptic scabies should be adopted, but some acari in the deeper part of the ear are very apt to escape treatment. The same precautions for preventing its introduction among rabbits should be observed as in sarcoptic scabies.

Mixed Cases of Sarcoptic and Psoroptic Scabies.—In addition to cases of pure sarcoptic infection and pure psoroptic infection, one frequently sees rabbits with both diseases. Out of eighteen rabbits suffering from scabies, six had a pure sarcoptic infection, three had a pure psoroptic infection, and nine were infected with both forms of parasite. The sarcoptes never infect the inside of the ears. In such mixed cases psoroptes only are found inside the ears, and a mixture of sarcoptes and psoroptes on the face, outside ears, neck, limbs, abdomen, and genitals. The rabbit at first has sarcoptic scabies on face, neck, etc.,

and psoroptic scabies inside its ears, and it is only after the diseases have lasted some time that the psoroptes spread from the ears over the body and a mixture of the two diseases is seen (Plate XXXVI. Figs. 8 and 9). One can usually tell the cases where there is a pure sarcoptic infection of the face and limbs from the mixed cases or pure psoroptic infection of the body. In the latter cases, there is always more crusting, more cracking of the skin, and more loss of hair than in the sarcoptic cases. Microscopically such mixed cases show an appearance very similar to that seen in pure sarcoptic scabies. The sarcoptes are more in evidence than the psoroptes, as they burrow more into the skin and so are not so apt to fall out of the sections.

Scabies in the Cat.

Two forms of scabies affect the cat—(1) A sarcoptic form due to *S. minor*; and (2) a chorioptic form due to *Chorioptes cynotis*.

Sarcoptic Scabies.—From a cat in the laboratory I obtained this parasite. The cat showed scaliness on the top of the head, outer aspect of the ears, on the face and neck. The skin there was scratched-looking but not very red. There was also patchy loss of hair. On the scaly areas abundant acari were found. These acari, as the name *S. minor* (Plate XXXIX. Fig. 26) indicates, are smaller than the *S. scabiei* (see Table), and very nearly approach in size to those found on the rabbit. The cat had been kept some time in the laboratory, and it is possible that it was infected from a rabbit. Neumann states that the identity of the rabbit and cat sarcopt has not yet been established. This form of scabies has been communicated to man but soon dies out.

Neumann also states that the *S. minor* of the cat is the cause of scabies in the rat. But although the appearance of the sarcoptes of the cat and rat are somewhat similar, the rat sarcopt is distinctly larger than that of the cat, and probably therefore is a different variety of *S. minor*.

Chorioptic Scabies of the Cat.—For a specimen of the parasite of this disease I am indebted to Professor Gofton. The chorioptes are found inside the ear. In the animal examined there was very little sign of irritation of the ear. The chorioptes are not unlike the psoroptes, and are arranged usually in pairs (Plate XL. Figs. 31, 32, and 33). They are smaller than the psoroptes, and are most readily distinguished from them by the short unjointed pedicle to the suckers on the legs, whereas, as already mentioned, the psoroptes have long, thin, jointed pedicles on the suckers. The chorioptes in the cat correspond very closely in size to those which I found in the ferret.

Mixed Cases.—The cat just referred to also had an infection of

the face and neck with sarcoptic scabies, so that, as in the rabbit, sarcoptic scabies and otacariasis may occur together in the same animal.

Ferret Scabies.

The ferret is attacked by two kinds of scabies: (1) A sarcopt, *S. scabiei*, var. *hydrochœri*, and (2) a choriopt, *C. cynotis*.

Sarcoptic Scabies.—I have examined three ferrets attacked with this disease. For one I am indebted to Professor Gofton, and for the others to Dr. J. P. M'Gowan. In all cases the feet were chiefly affected. On the pads of the feet and between the claws were large masses of crusts in which sarcoptes were very numerous (Plate XLI. Fig. 34). There was also some crusting on the rims of the ears (Plate XLI. Fig. 35), all along the tail (Plate XLI. Fig. 38), and around the genitals and anus. The body was not affected. The crusting and swelling in the feet were so great that the animals had difficulty in walking. They frequently tore at their feet with their teeth. They were not in good condition, their fur stood on end, and, as Neumann describes, their bodies were covered with a thick viscid sweat which smelt strongly of their characteristic odour.

How the ferrets contract the disease is doubtful. Naturally, what suggests itself is an infection from the rabbit, but this cannot be, as the acarus here is not *S. minor* as usually found in the rabbit, but *S. scabiei*. Sarcoptes from the ferrets were placed on the skin of two healthy rabbits. Pieces of the skin from the soles of the feet and tails of the ferrets containing very numerous sarcoptes were also placed in these rabbits' cages, but the rabbits remained unaffected. Friedberger and Fröhner (1908[5]) state that rabbits suffering from *S. scabiei* may infect ferrets, but Neumann does not mention that form of scabies as occurring in rabbits.

In all three ferrets examined the same kind of sarcopt was found. It does not correspond in size to any of the other forms of sarcoptes which I examined. Neumann quotes Raillet as stating that the ferret probably gets the disease from the pole-cats which are frequent in rabbit warrens, but the cat sarcopt is *S. minor* and not *S. scabiei*. The disease is not known to be transmitted from the ferret to man. Sections made from the ears and tails of the ferrets (Plates XLI. and XLII. Figs. 39 and 40) show very abundant acari embedded deeply in the horny layer of the skin. The whole horny layer is thickened and the crusts and scales are all tunnelled by the passage of the acari. There is some dilatation of the vessels of the corium, and an infiltration of small round cells which are extraordinarily scanty considering the number of acari on the surface. Nowhere were vesicles to be seen in the epithelial layers such as are seen in the human skin in scabies.

Chorioptic Otacariasis of the Ferret, due to *C. auricularum*,

var. *furonis*.—Two of the ferrets examined, in addition to having sarcoptes on the feet and tails, had chorioptes inside the ears. These were found, as already stated, to be very similar in size and appearance to the chorioptes of the cat. They were very numerous inside the ears, and, beyond a slight scaliness, caused no change in the skin. Neumann states that they frequently cause destruction of the tympanic membrane and lead to otitis media, caries of the temporal bones, and meningitis. They do not, as in the dog and cat, cause any epileptiform attacks.

Chorioptes from these ferrets were placed inside the ears of two rabbits, but the result was negative.

Fox Scabies.

Professor Gofton also kindly supplied me with material from a case of scabies in a fox. Scales, etc., removed from this fox contained very numerous sarcoptes and their ova, which do not differ except in size from the sarcoptes of the other animals. The females are very nearly of the same size as the sarcoptes obtained from the rats, but of the males are somewhat larger.

Rat Scabies.

Scabies in rats differs very markedly in appearance from the scabies of the other animals which have been dealt with. The ears, nose, feet and tail are affected, and in some instances the genitals also. The ears show small hard warty-looking growths (Plate XXXVIII. Fig. 18). These growths are chiefly at the edge of the auricle, but also to a less extent on the inner and outer surfaces of the ear (Plate XXXVIII. Figs. 19 and 21). On the tip of the nose a horny mass, often of considerable length, frequently develops like the horn on the nose of a rhinoceros. If this horny mass gets knocked off it soon regrows. The tail in its whole length is scaly and covered with similar but smaller warty growths. Less frequently the legs and genitals are affected also, and in some cases the hair of the neck and back falls out, but I have never found any acari on the neck or back. From all the warty lesions very abundant sarcoptes are easily obtained. They are round in shape, and show the usual characters of sarcoptes (Plate XXXVIII. Fig. 20). In size they are very similar to those found on the fox, *i.e.* they are intermediate in size between the *S. scabici* of man and the ferret and the *S. minor* of the cat and rabbit. When first I saw this disease on rats, scabies never occurred to me. The condition was obviously contagious, but it looked more like some form of contagious wart than a disease due to an animal parasite. My attention was first drawn to its true nature by an article by Schurmann (1908 [3]). If these warty masses are examined in liquor potassæ sarcoptes and their

ova are easily seen. The removal of the mass leaves a red moist oozing surface beneath. The eruption itches, but does not affect the health of the animals much. It never seems to spread beyond the parts already indicated.

If the skin be examined microscopically (Plate XXXIX. Fig. 23) adult sarcoptes are found embedded in the horny layer (Plate XXXIX. Figs. 22 and 24). The acarus lies in a depression on the swollen tissues, which rise upwards and surround it. It lies in the horny layer, but, in places, the Malpighian layer is hypertrophied; in others it has completely disappeared, forming an ulcer, and the acarus is in contact with the cell infiltration in the corium. Schürmann describes the acarus as sometimes penetrating deeply into the deep-lying connective tissue and even as far as the cartilage, but in the micro-photograph which he gives the acarus is obviously surrounded by epithelium, and is simply lying in a depression on the surface. There being very little distance between the surface epithelium and the cartilage, the animal appears to have penetrated to the cartilage. All the corium under the place where an acarus is situated is filled with a dense infiltration of small round cells, and probably the greater part of the cell infiltration and any ulceration which may result is due to the mechanical irritation and pressure produced by the animal pulling on the horny masses in scratching and not to any destruction of the epithelium by the acarus itself.

The disease spreads rapidly from one rat to another. Ascher gives three to ten days as the time it takes to develop. It is spread by direct contact, or by infected cages, etc. Ascher (1910 [4]) put rats in a cage where diseased animals had been kept and they soon developed the disease.

Into a cage containing two healthy mice the skin of a rat with typical sarcoptic scabies was placed and left for some days, but after an interval of a month neither of the mice showed any signs of the disease. On two different occasions considerable quantities of material containing living sarcoptes and their ova from the ears of rats were placed in the nest beside the mice, but with no result. Therefore, from the fact that the mice in the laboratory, although living in and near cages where rats with sarcoptic scabies were or had been, did not develop the disease, and from the fact that inoculation from diseased rats had no result, and also from the appearance of the sections from a mouse's ear to be described later, one must conclude that mice are probably not susceptible to the sarcoptic scabies of the rat.

In order to test the infectiveness of rat scabies for the rabbit, a rat with marked scabies was placed in the same cage as a healthy rabbit. The rat usually slept across the back of the neck of the rabbit, the rabbit's ears lying back over the top of the rat. In this way they came into very close and prolonged contact, and although these

animals lived together for more than three months the rabbit never contracted the disease.

These results are similar to those obtained by Schürmann and Ascher, who also failed to inoculate mice and rabbits from the rat. Schürmann also tried to inoculate dogs and guinea-pigs from the rat, but without result.

The same two observers report that the man who attended to their rats developed an eruption on the back of the hands due to an infection with rat sarcoptes which were found on the hand. It got well in a short time.

The treatment of rat scabies is simple. Dr. Ford Robertson, Pathologist to the Scottish Asylums Laboratory, informs me that he has eradicated the disease by putting the rats among abundant straw, by rubbing themselves through which they clean themselves.

Scabies in Mice.

As scabies among rats was so prevalent, one naturally made investigation to try and find out whether it also occurred among mice. In the laboratory it has never been noticed affecting the mice, although mice have frequently been put in cages where rats suffering from scabies had previously been kept. On inquiry, however, Dr. Ford Robertson informed me that some time ago several of the mice with which he was working had shown warty-looking growths on the ears and about the head. The disease, however, had now disappeared from the mice, so that unfortunately I did not see any of the living animals showing it. Dr. Ford Robertson, however, kindly gave me a mouse which had been preserved in formalin for some months. This animal presented one small crusted warty-looking lesion on the rim of the ear, not unlike the lesion observed on the rat's ears. The affected part of the ear was put through paraffin and serial sections were cut.

The sections show here and there, lying on the surface of the skin, parasites which differ somewhat from the sarcoptes observed on the rat's ears. They are larger than the sarcoptes, and not so rounded in shape. On the other hand, they are not so large as the psoroptes seen on the rabbit's ear. They lie on the surface of the skin and are not embedded at all in the epithelium. That fact alone is sufficient to exclude this parasite from the group of sarcoptes which are characterised by their burrowing in the horny layer of the skin. There is no sign of any change in the skin subjacent to these parasites, but at a short distance from the acari there is an area of very marked inflammation of the skin. The whole of the skin is infiltrated with small round cells right down to the subcutaneous tissue. The surface of this area shows a complete disappearance of the epithelium, and is covered with a thick crust of dried exudate and leucocytes. In this crust there are no signs of acari or of their faeces. Very

TABLE.

Host.	Kind of Parasite.	Sex of Parasite.	Diameter in Millimetres.	1	2	3	4	5	6	7	8	9	10	Average.
MAN	*Sarcoptes*	Female	Long	0·432	0·448	0·448	0·432	0·448	0·448	0·400	0·448	...	...	0·438
	(*ovigerous*)		Broad	0·352	0·336	0·352	0·320	0·352	0·352	0·304	0·352	...	...	0·340
,,	,,	Male	Long	...	..	...	...	...	...	...	...	...	...	...
			Broad	...	..	...	...	...	...	...	...	...	...	..
RABBIT	*Sarcoptes*	Female	Long	0·208	0·208	0·208	0·208	0·224	0·224	0·224	0·240	0·208	0·240	0·215
			Broad	0·176	0·160	0·160	0·176	0·176	0·176	0·176	0·208	0·160	0·208	0·177
,,	,,	Male	Long	0·160	0·160	0·144	0·160	...	...	...	...	...	...	0·156
			Broad	0·112	0·128	0·128	0·128	...	...	...	...	...	...	0·124
CAT	*Sarcoptes*	Female	Long	0·224	0·208	0·208	0·176	0·224	0·240	0·224	0·224	0·240	0·224	0·219
			Broad	0·176	0·160	0·160	0·160	0·176	0·176	0·176	0·176	0·176	0·176	0·171
,,	,,	Male	Long	0·144	0·144	...	...	...	...	...	...	...	...	0·144
			Broad	0·128	0·128	...	...	...	...	...	...	...	...	0·128
FOX	*Sarcoptes*	Female	Long	0·320	0·320	0·288	0·336	0·336	0·320	0·336	0·336	0·352	0·336	...
			Broad	0·224	0·240	0·224	0·240	0·240	0·240	0·256	0·256	0·240	0·288	...
,,	,,	,,	Long	0·336	0·336	0·352	0·288	0·352	0·320	0·320	0·352	0·352	0·352	0·332
			Broad	0·224	0·240	0·256	0·240	0·224	0·224	0·240	0·256	0·240	0·272	0·243
,,	,,	Male	Long	0·208	0·192	0·208	...	...	...	...	...	...	...	0·202
			Broad	0·160	0·160	0·160	...	...	...	...	...	...	...	0·160
FERRET	*Sarcoptes*	Female	Long	0·368	0·384	0·400	0·400	0·400	0·400	0·352	0·400	0·400	0·368	0·387
			Broad	0·288	0·304	0·304	0·304	0·304	0·320	0·272	0·288	0·288	0·272	0·294
,,	,,	Male	Long	0·208	0·208	0·208	...	...	...	...	...	...	...	0·208
			Broad	0·160	0·160	0·176	...	...	...	...	...	...	...	0·165
RAT	*Sarcoptes*	Female	Long	0·320	0·336	0·368	0·336	0·304	0·336	0·320	0·336	0·320	0·336	0·331
			Broad	0·240	0·256	0·288	0·288	0·256	0·256	0·256	0·272	0·272	0·272	0·265
,,	,,	Male	Long	0·144	0·160	0·160	0·160	...	...	...	...	...	...	0·156
			Broad	0·128	0·128	0·128	0·112	...	...	...	...	...	...	0·124
RABBIT	*Psoroptes*	Female	Long	0·432	0·368	0·496	0·448	0·512	0·480	0·448	0·480	0·400	0·384	0·444
	(*pubescent*)		Broad	0·368	0·304	0·352	0·352	0·304	0·368	0·336	0·320	0·352	0·256	0·330
,,	,,	Female	Long	0·540	0·720	0·624	0·640	0·624	0·672	0·704	0·800	0·768	0·672	0·684
	(*ovigerous*)		Broad	0·400	0·432	0·384	0·400	0·400	0·400	0·416	0·480	0·464	0·416	0·419
,,	,,	Male	Long	0·400	0·464	0·480	0·432	0·512	0·464	0·480	0·512	0·480	0·432	0·465
			Broad	0·304	0·304	0·336	0·304	0·304	0·320	0·320	0·352	0·352	0·288	0·318
CAT	*Chorioptes*	Female	Long	0·288	0·336	0·320	...	...	...	...	...	...	...	0·314
	(*pubescent*)		Broad	0·208	0·208	0·224	...	...	...	...	...	...	...	0·213
,,	,,	Female	Long	0·448	0·464	0·448	0·400	0·448	0·448	0·432	0·416	0·400	0·416	0·432
	(*ovigerous*)		Broad	0·256	0·272	0·256	0·240	0·272	0·288	0·288	0·256	0·240	0·240	0·260
,,	,,	Male	Long	0·288	0·320	0·336	0·288	0·304	...	...	...	...	...	0·307
			Broad	0·224	0·240	0·240	0·224	0·240	...	...	...	...	...	0·233
FERRET	*Chorioptes*	Female	Long	0·400	0·336	...	...	...	...	...	...	...	...	0·368
	(*pubescent*)		Broad	0·256	0·208	...	...	...	...	...	...	...	...	0·232
,,	,,	Female	Long	0·400	0·368	0·416	0·432	0·416	0·432	0·432	0·448	0·416	0·400	0·416
	(*ovigerous*)		Broad	0·256	0·240	0·256	0·256	0·256	0·256	0·272	0·272	0·240	0·240	0·254
,,	,,	Male	Long	0·320	0·320	0·288	0·288	0·288	0·304	0·320	0·320	0·320	0·320	0·308
			Broad	0·224	0·240	0·256	0·256	0·240	0·240	0·240	0·240	0·240	0·240	0·240

numerous colonies of staphylococci are visible in and below the crusts. These lesions are apparently of the nature of small foci of suppuration, and probably due to inoculation of the skin with pyogenic organisms by scratching. As no further material could be obtained one could not say from the sections alone that the parasite was an acarus. It is certainly not the sarcopt seen in rats.

The foregoing table of measurements was made to show the relative size of the acari from the different animals compared with the human acarus. The measurements in fractions of a millimetre were made from several acari from different animals suffering from the same disease, and the average taken for each variety of acarus. What strikes one most about these measurements is the wonderful uniformity in size of acari of the same kind. The largest sarcoptes are the human; next come those from the ferret. Both these are varieties of the *S. scabiei*. Then come the fox, rat, cat, and rabbit sarcoptes, all in order of size. The cat and rabbit sarcoptes belong to the class known as *S. minor* and are very similar in size, whilst the sarcoptes of the fox and rat are very nearly of equal size and are intermediate between the *S. scabiei* and the *S. minor*. The small size of all these sarcoptes makes it almost impossible to see them with the naked eye amongst the scales, etc. in which they lie. The psoroptes and chorioptes, however, can be easily recognised with the naked eye. The chorioptes of the cat and ferret are practically identical in size and microscopic characters.

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DESCRIPTION OF PLATES XXXIV.-XLII.

Plate XXXIV.

Fig. 1.—Rabbit suffering from sarcoptic scabies.

Fig. 2.—Section of skin from case of sarcoptic scabies in the rabbit. It shows acari embedded deeply in the horny layer of the skin. (×60.)

Fig. 3.—Female sarcoptes from rabbit. (×150.)

Fig. 4.—Section of ear of rabbit suffering from psoroptic scabies showing layers of crusts filling up interior of ear. Note very small amount of cell exudate in corium. (×5.)

FIG. 5.—Section of skin from case of sarcoptic scabies in the rabbit, showing acarus in horny layer of skin and also the spaces left in the crusts on surface by the acari tunnelling their way through. (×50.)

PLATE XXXV.

FIG. 6.—Rabbit suffering from psoroptic scabies inside ear and sarcoptic scabies on body, etc. Note distribution of eruption on face, legs, etc.

FIG. 7.—Rabbit showing psoroptic scabies inside ear. Note laminated character of crusts.

PLATE XXXVI.

FIG. 8.—Rabbit showing advanced psoroptic scabies inside ear and early sarcoptic scabies around eye.

FIG. 9.—Rabbit showing mixed psoroptic and sarcoptic scabies.

FIG. 10.—Rabbit showing pure psoroptic scabies.

FIG. 11.—Psoroptes (male and female) from inside of ear of rabbit. (×30.)

PLATE XXXVII.

FIG. 12.—Psoroptes from inside of rabbit's ear. (×20.)

FIG. 13.—Psoroptes from inside of rabbit's ear, male. (×40.)

FIG. 14.—Psoroptes from inside of rabbit's ear, female. (×50.)

FIG. 15.—Rabbit showing psoroptic scabies of face.

FIG. 16.—Section of skin from ear of rabbit with psoroptic scabies. Note hypertrophy of Malpighian layer of skin at places, with parakeratosis of horny layer. At places there is almost complete disappearance of the epithelial layers. The corium is filled up with a dense infiltration of small round cells.

PLATE XXXVIII.

FIG. 17.—Ova, etc. from ear of rat suffering from sarcoptic scabies. Note young acari just hatched, unhatched ova and also empty egg cases. (×50.)

FIG. 18.—Rat showing sarcoptic scabies. Note lesions on tip of nose, ears, legs, and tail.

FIG. 19.—Rats with sarcoptic scabies. Note warty growths on ears.

FIG. 20.—Female sarcopt of rat. (×210.)

FIG. 21.—Slightly enlarged view of warty growths on rat's ears in sarcoptic scabies.

PLATE XXXIX.

FIG. 22.—Section of ear of rat with scabies. Space seen where acarus has dropped out. Note depression of epithelium with ulceration at one place; also marked small-celled infiltration of corium. (×100.)

FIG. 23.—Sections of rat's ear with scabies. Note sarcopt embedded in depression on the surface. (×25.)

FIG. 24.—High-power view of same as last, showing acarus and cell infiltration. (×100.)

FIG. 25.—Head of guinea-pig infected with sarcoptic scabies from the rabbit.

FIG. 26.—Female sarcopt from a cat. (×60.)

FIG. 27.—Female sarcopt from a human being. (×70.)

FIG. 28.—Female sarcopt from a fox. (×90.)

PLATE XL.

FIG. 29.—Guinea-pig infected with sarcoptic scabies from a rabbit.

FIG. 30.—Section of skin from mouse's ear. Scabies? (×10.)

FIG. 31.—Chorioptes, male and female, from ear of cat. (×60.)

FIG. 32.—Chorioptes from ear of cat, male. (×70.)

FIG. 33.—Chorioptes from ear of cat, female. (×70.)

PLATE XLI.

FIG. 34.—Slightly enlarged view of ferret's foot showing sarcoptic scabies.

FIG. 35.—Slightly enlarged view of ferret's ear showing sarcoptic scabies.

FIG. 36.—Sarcoptes from ferret, male. (×120.)

FIG. 37.—Sarcoptes from ferret, female. (×120.)

FIG. 38.—Tail of ferret with sarcoptic scabies.

FIG. 39.—Section of ear of ferret showing sarcoptes embedded in horny layer of skin, and tunnelling of the crusts by acari. (×20.)

PLATE XLII.

FIG. 40.—Section of tail of ferret showing same as last. (×30.)

FIG. 41.—Section of ear of mouse showing acarus? (×20.)

FIG. 42.—Chorioptes (female) from ear of ferret. (×100.)

FIG. 43.—Chorioptes (male) from ear of ferret. (×100.)

FIG. 1.

FIG. 2.

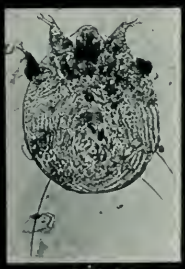
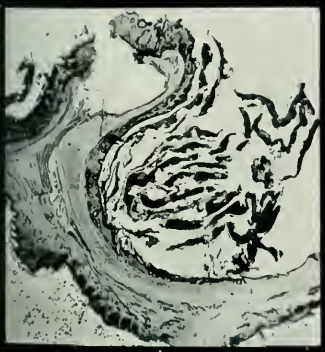


FIG. 3.



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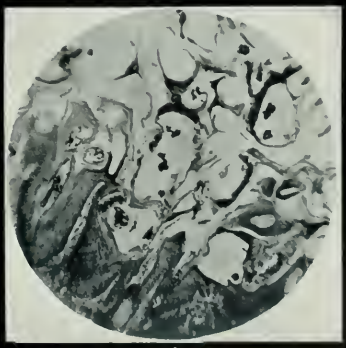

FIG. 5.

FIG. 6.

FIG. 7.

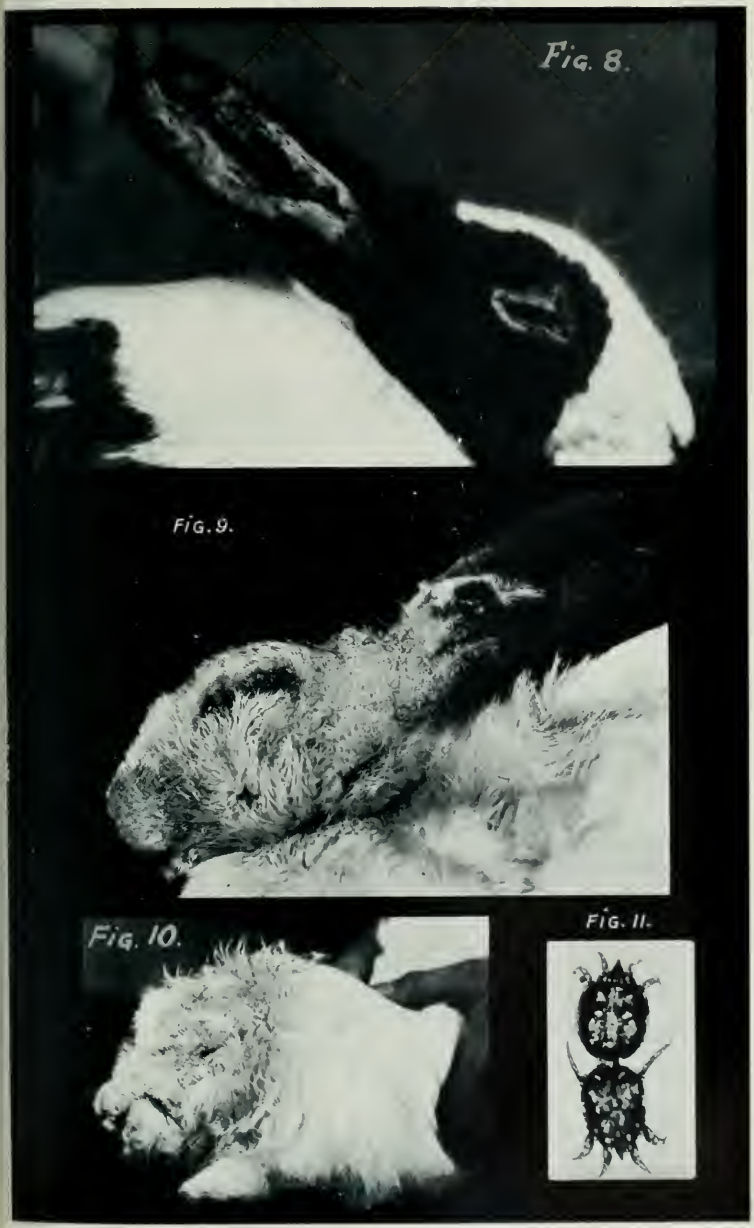
Fig. 8.
Fig. 9.
Fig. 10.
Fig. 11.

Fig. 12.

Fig. 13.

Fig. 14.

Fig. 15.

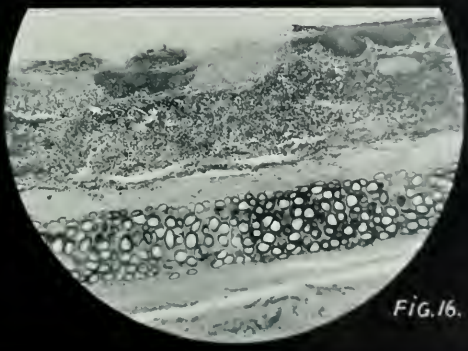

Fig. 16.

FIG. 17.

FIG. 18.

FIG. 19.

FIG. 20.

FIG. 21.

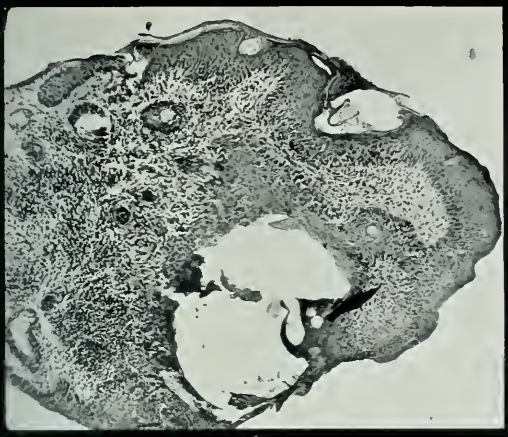

FIG. 22.

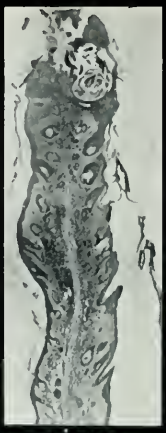

FIG. 23.

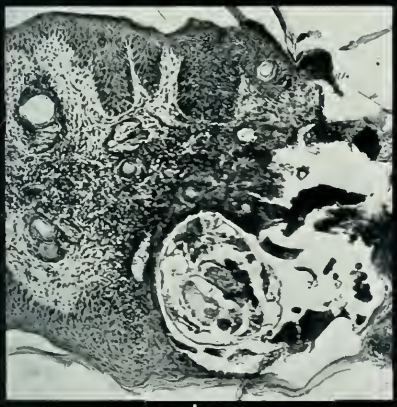

FIG. 24.

FIG. 25.

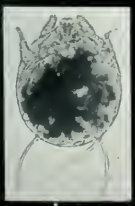

FIG. 26.

FIG. 27.

FIG. 28.

FIG. 29.

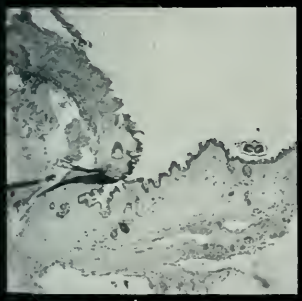

FIG. 30.

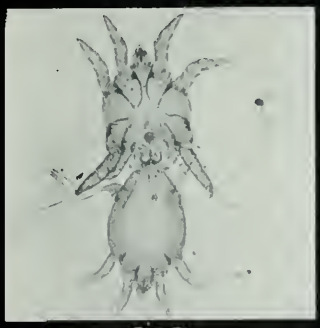

FIG. 31.

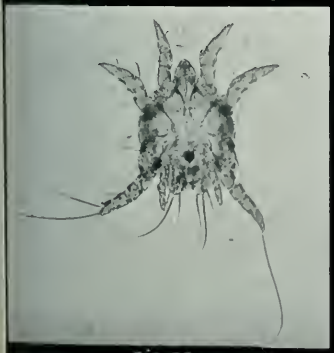

FIG. 32.

FIG. 33.

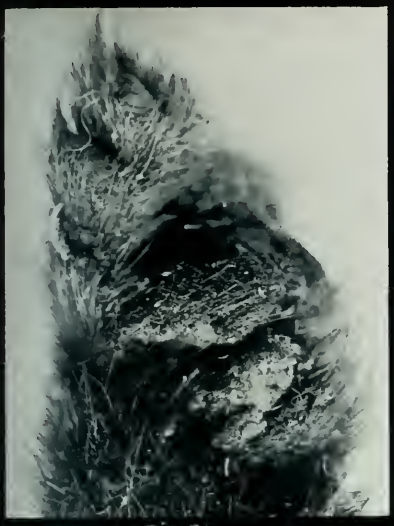

FIG. 34.

FIG. 35.

FIG. 37.

FIG. 36.

FIG. 38.

FIG. 39.

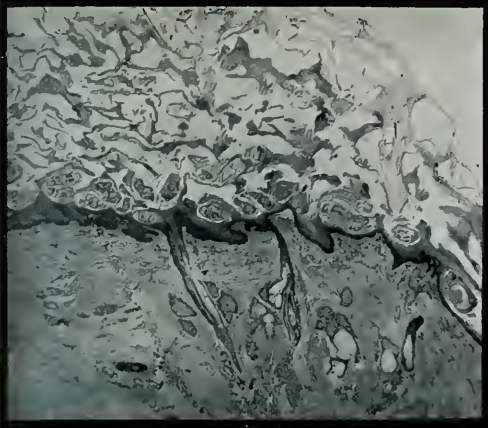

FIG. 40.

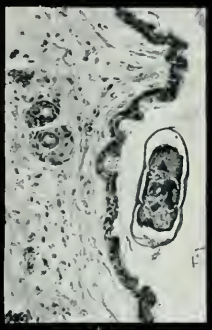

FIG. 41.

FIG. 42.

FIG. 43.

Fungus Infections of the Finger-Nails.

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FUNGUS INFECTIONS OF THE FINGER-NAILS.[1]

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In his handbook of *Diseases of the Skin* (1907), Whitfield states that fungus infections of the nails are probably commoner than is generally supposed. That statement is borne out by the fact that within the last three years I have had the opportunity of observing no fewer than 19 cases; 17 of these were seen as out-patients in the Skin Department of the Royal Infirmary under Dr. Norman Walker's care, and to him I am indebted for permission to record them. For one case, which was an out-patient at Leith Hospital, I am indebted to Dr. Langwill.

I do not propose to describe each case separately. They fall into three groups, viz.—(1) Ringworm of the nails; (2) Favus of the nails; (3) a Fungus Infection of the nails of unknown origin.

As the cases of ringworm form the most important group, they will be dealt with first. There were 16 of these ringworm cases, 14 of which belonged to Edinburgh and 2 to Leith.

A curious fact was the predominance in the female sex—only 2 being in males. This predominance is difficult to explain. Török says that tinea unguium is commoner in those who manicure the nails, and probably injure them in so doing, but the majority of our patients were not of the class which bestows much care on the nails. It is possible that the frequent washing of infected material, *e.g.* clothes, &c., may be the reason why females so frequently suffer.

Ringworm of the nails may occur at any period of life. The youngest of my cases was 11, and the oldest 58 years of age. The majority were girls of between 20 and 30 years. This age-incidence corresponds more nearly to that of tinea corporis than of tinea capitis.

Ringworm of the nails is very rare in children with ringworm of the scalp. Sabouraud, in l'Ecole Lailler, where there were always about 300 children with ringworm of the scalp, in four years saw only 2 cases where the nails were affected. This infrequency of nail infection in children with ringworm may be partly

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due to the fact that ringworm of the nails is always due to the large-spore fungus, but even amongst children suffering from large-spore tinea of the scalp nail affections are very rare. I think the most probable explanation is, that because tinea capitis so rarely causes any itching there is no tendency to scratch and so infect the nails. The inflamed ringworms (kerion) are also too tender to allow of scratching.

Source of Infection.—The source of infection was difficult to trace in many of the cases. In only 1 of the 16 cases did the patient suggest that the disease was due to an infection from anyone else. The patient blamed the washing of clothes worn by her husband and son, who had what was apparently tinea barbæ. In all the other cases the patient had no idea of the condition being a contagious one. In another case there is also little doubt that the disease was contracted from her brother who, I know, had tinea barbæ at the time when her nails became infected, but as cultures were not made from the brother's beard I cannot say whether or not both conditions were due to the same fungus.

Two of the cases were husband and wife and other two were sisters, and in these cases there was probably either a common source of infection or infection the one from the other.

One woman was undoubtedly infected from her children, who had tinea capitis of large-spore variety. In washing and rubbing their heads she contracted ringworm of the hands and fingers, the disease later spreading to the nails. In this case cultures showed that the same fungus was present in the nails as on the children's heads. This method of infection has been previously described by Vidal, who noticed that nurses who epilated ringworm cases at the Hôpital St. Louis in Paris frequently became infected with ringworm of the nails.

In all the other cases absolutely no source of infection could be traced either from previous lesions on their own skins, from infection from their relatives or friends, or from animals. Each patient was particularly questioned with regard to these points.

In two cases there was a history of injury to the nail shortly before the disease began, and it is possible that infection may have taken place at that time.

One case was associated with spoon-nails (coilonychia). The spoon-nail was well marked in all the nails, and possibly such nails are more liable to fungus infections than normal ones.

Duration.—The duration of the disease varied from three weeks up to seven years, the average time being about four years. Only

a very few of the cases had been previously treated, and in some the disease was discovered accidentally, when the patient came complaining of some other condition.

Extent.—The number of nails affected in each case varied from one up to seven. No one nail was more often affected than another. In one-half of the cases both hands were affected, in the other half only one hand. In the 8 cases of the latter group, 4 showed the disease on the right hand and 4 on the left. In none of the cases were the toe-nails affected.

Diagnosis.—The diagnosis in all cases was comparatively easily made, the appearance of the nails being very constant. In all previously recorded cases the authors lay stress on the splitting up and splintering off of the free margin of the nail. This is characteristic of the disease, but as the patients often clip away any loose parts, the nail frequently does not show this to any great degree. The colour of the nails has been variously described from a yellow-white to a brown or black colour. In nearly all my cases I have found that there are definite zones presenting different colours and appearances, these zones being very constant in arrangement. The infection seems to take place from the under surface of the free margin of the nail. At the tip the nail becomes undermined, dark in colour, and softer than normal, so that if a blunt instrument be inserted under it some of the softened nail substance can be easily removed. The disease slowly spreads up the nail, and as it spreads the nail becomes more broken up, till finally, when the whole of it is infected, the free margin breaks off in splinters of a dirty white colour. These broken parts at the tip are easily removed. Then comes a zone where the nail is light yellow in colour, longitudinally striped with darker lines. At this level pieces of the nail can be scraped off only with difficulty. Further up still there is a peculiar dark yellowish-red zone, fairly smooth on the surface, and showing very little tendency to split up. These three zones were distinctly seen in most of the affected nails, and appear to me to be very characteristic of the condition, more especially the yellow-red zone at the upper part. This yellow-red smooth area represents the spreading edge, where the infection is only just beginning. In the light yellow area further down, the nail is permeated with the fungus, but not yet sufficiently so as to cause the nail to break up. The tip is the part of the nail which has been longest infected, is thoroughly permeated by the fungus, and so is split up and cast off. These zones look as if they were stationary, whereas in reality the upward growth of the fungus is simply keeping pace with the downward growth of the nail.

In addition to these colour changes and splitting up, there is also in many cases a heaping up of the nail into irregularly arranged prominences with dark blackish-brown depressions between. When the nail remains flat the nail substance is probably the only part affected with the fungus, but when it becomes raised up and distorted the nail-bed is probably also affected. In many cases the lunule has disappeared altogether, and the tissues at the base of the nail are red and somewhat swollen. In such cases the nail is very distorted, and it is an indication probably that the growing nail matrix is invaded by the fungus. Apart from the disfigurement there are no symptoms associated with the disease.

Microscopic Appearances.—In all the cases the diagnosis was confirmed by microscopic examination. Pieces of the nail were scraped off with a sharp spoon or clipped off with scissors, put in liquor potassæ (6 per cent.) and gently heated for a few minutes. They all showed the same appearances. Inside the nail substance branching mycelium and spores are to be seen. The spores are square in shape and arranged very characteristically in rows. If the fungus is not visible at once the pieces of nail should be left in the caustic potash over night, and if it is present at all there is no difficulty in seeing it next morning. In some of the cases fungus was not found in the first pieces of nail examined. Therefore in all cases of suspected fungus disease of the nails scrapings should be repeatedly examined. I would also strongly recommend that in every case where a patient presents a splitting up of the nails, which does not affect all of them, that a thorough examination be made for the presence of fungus. Finding of the typical fungus is proof positive of ringworm, but the failure to do so on first examination does not necessarily exclude that disease.

An attempt was made to stain the fungus in the nails by gentian violet and other stains, but the results were not satisfactory. For all practical purposes the caustic potash method is quite sufficient.

Cultures.—In order to determine the kind of fungus present cultures were made from the nails of 8 of the patients. Pieces of nail were scraped or clipped off and placed for 2 or 3 minutes in absolute alcohol. Whilst in the alcohol they were broken up into as small pieces as possible. These were allowed to dry for a few minutes and then implanted on Sabouraud's proof medium, which contains 4 per cent. maltose, 10 per cent. peptone, and 1·5 per cent. agar. The cultures were kept at a temperature of 20° C. Some

of them showed a pure culture of the fungus, but the majority contained also staphylococci, various yeasts, and penicillium glaucum, but by early subculture the fungus was easily isolated. In 5 of the cases trichophyton crateriforme flavum (primrose coloured) was obtained. These cultures are the same as that described by Colcott Fox in cases of ringworm of the body and scalp in London. This culture begins as a white downy growth, which very soon shows a central depression and the characteristic sulphur yellow colour. The central depression enlarges till a typical crater is formed. In some there are radiating depressions running outwards from the crater in the centre, in others these are not marked. The surface of the culture looks powdery, and in old cultures the centre crater area becomes brownish in colour and cracked. If these cultures are kept for some time a change takes place. There appears a whitish growth, which gradually spreads over the yellow culture and obscures it. This is the pleomorphic change which occurs in all old ringworm cultures which are grown on media containing sugar, such as maltose. Subcultures from this altered culture give very beautiful whitish-yellow growths which are distinctly crateriform.

The cultures from other two of my cases correspond to the trichophyton rosaceum, which is the large-spored fungus found on birds. The young cultures are fluffy and white, but soon show a delicate pink colour, and in some of the growths a yellowish tinge also in the centre. They are raised in the centre and have a few radiating depressions. These cultures also rapidly tend to show a pure white pleomorphic growth.

From another case peculiar cultures were obtained. At first they were contaminated by staphylococcus citreus. They were repeatedly subcultured and a growth obtained which had a pink tinge in places. On making subculture from the white and pink parts two distinct cultures were obtained. Unfortunately the growth of these cultures was not watched very closely, and I cannot say definitely whether the culture was originally white all over or whether there was a mixture of pink and white from the first. The white culture corresponds somewhat to the trichophyton accuminatum in its appearances, but is smoother on its surface. Until I have watched its growth by further subculture I am not prepared to say whether it is the trichophyton accuminatum or a pleomorphic form of the pink culture.

So far as I can find from the literature, Sabouraud and Favera are the only two observers who have previously investigated the

kinds of cultures obtained in ringworm of the nails. Sabouraud found trichophyton accuminatum and trichophyton violaceum; Favera obtained trichophyton violaceum and trichophyton rosaceum. To these must now be added the trichophyton crateriforme flavum found in my cases.

Treatment.—The treatment in these cases was difficult. Many of them were only seen once, so that I do not know whether the treatment recommended was ever carried out or not. All methods of treatment have for their object the softening and mechanical removal of as much of the diseased nail as possible and the application of some continuous antiseptic dressing.

The easiest method is to keep the nail covered with an india-rubber finger-stall after applying ung. hydrarg. ammoniat. night and morning. Of course the softened nail must be scraped away every day. One of the cases was cured by this method in 3 months, but in the majority of cases that method is quite ineffectual. A better method is Harrison's, *i.e.* in addition to the regular daily scraping a solution of pot. iodid., ʒj. in liq. potassæ, and aq. destill., āā ʒss., is applied for half an hour on a piece of cotton-wool. It is then removed, and for the rest of the 24 hours a solution of hydrarg. perchlor., grs. iv. in spir. vini, and aq. destill., āā ʒss., is applied on lint and the finger covered with an india-rubber finger-stall. These applications are repeated daily till the nail becomes black and so soft that it can be removed. In one case a cure was effected by the above method in 2 months. The reason for the failure in the other cases in which it was tried was that the patients stopped the applications as soon as the nails became the least painful. To get a good result the patient must be seen frequently, and the nails scraped by the doctor himself.

A new method which has given encouraging results is one devised by Dr. Norman Walker. He applies Fehling's test solution on lint to the nail, covers it with a finger-stall, and leaves it on for a day or two. At the end of that time the whole nail is so soft and pulpy that it can easily be removed by forceps. After the nail is removed a solution of copper sulphate, grs. 10 in water ʒj., is used as a continuous dressing. With this method good results were obtained in some of the cases; but whatever method is used long and painstaking treatment is essential for a successful result. When the disease is cured the nail grows in again without any deformity.

Favus Cases.—I have only seen 2 cases of favus of the nails. These were both in children with favus of the scalp. The appear-

ance of the nails is very similar to that of tinea, but the nails are yellower in colour. A mass of fungus also tends to accumulate like a scutulum underneath the nail and raise it up. The diagnosis is made by finding the fungus mieroseopically, and the treatment the same as in ringworm.

The other fungus infection which I wish to mention occurred in a girl aged 22, in whom 3 nails had been diseased for 10 years. The nails were greenish-yellow in colour and splintering off. There was also some heaping up of the nail at plaees. Serapings from the nails showed that fungus was present, but it differed altogether from the fungus seen in the ringworm and favus cases. The myeelium was very fine, and in long slightly branched threads. The spores were arranged in bunches, and were round or pear-shaped. Cultures gave a fluffy greyish-white growth. This culture is certainly not a ringworm or favus, but without further observation I am not prepared to say to what class of fungus this one belongs. The nails were removed in this case after being softened by caustic potash, but some weeks later the new growing nail was still found to be infected with the fungus.

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Plate I.

Ringworm of the finger-nails.

Plate II.

Ringworm of the finger-nails.

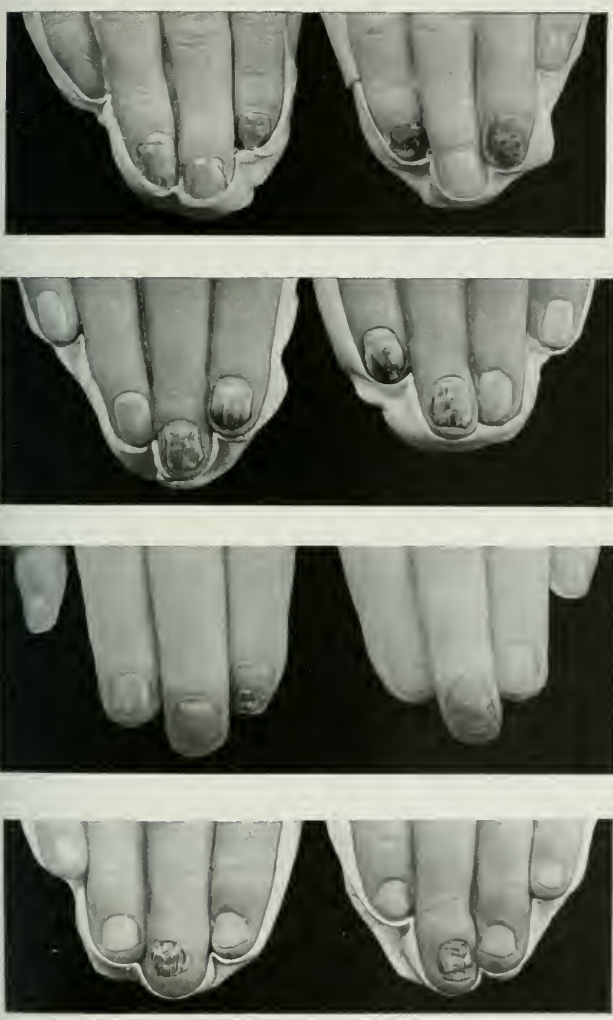
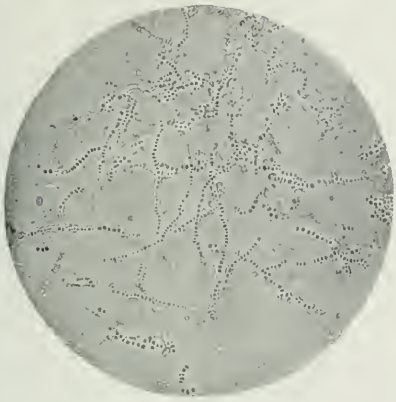


PLATE III.

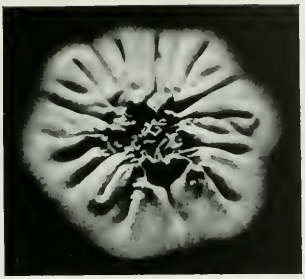


Ringworm fungus in finger-nail. Mounted in liquor potassæ. (× 400.

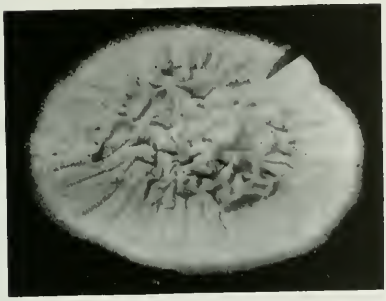
Same as above. (Oil immersion.)

Plate IV.

Cultures (about six weeks old on Sabouraud's medium) of fungi from finger-nails.



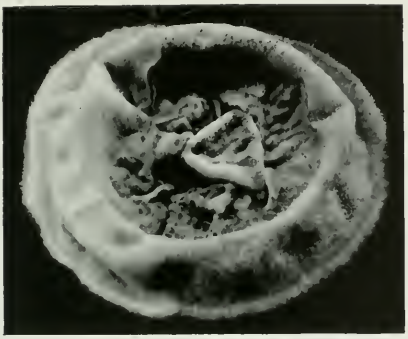
Trichophyton accuminatum.



Trichophyton rosaceum.

Trichophyton crateriforme flavum.

Plate V.



Trichophyton crateriforme flavum.

Pleomorphic form of trichophyton crateriforme flavum.

PLATE VI.

Fungus (unknown variety) in finger-nail.

Fungus from finger-nail (variety unknown.) Grown for six weeks on Sabouraud's medium.

ON AN OUTBREAK OF SCARLET FEVER IN A SMALL SCOTTISH BURGH, ASSOCIATED WITH MILK, THE COWS SUPPLYING WHICH WERE AFFECTED WITH A TEAT ERUPTION.

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HAVING regard to the excellent—and, we think, on the whole, fair—résumé of the literature on this subject by Professor Gofton, read at the meeting of the Association of Veterinary Officers of Health, in Glasgow, on September 29th, 1910, and published in the *Veterinary Journal* for October, 1910, we consider it unnecessary to deal here at greater length with the literature, and would refer those interested to his paper. Our reason for publishing a note on this outbreak is that the eruption seems to have appeared on the teats of the cows some time *after* the outbreak of scarlet fever had begun. In most of the epidemics recorded the eruption existed on the cows' teats for some time previous to the outbreak of scarlet fever; this epidemic being exceptional in that respect, we thought it worth recording.

In the spring of the present year an outbreak of scarlet fever arose in a small industrial

burgh of some two thousand inhabitants in Scotland. There were eighty-one cases in all, of whom one died. Of the cases sixty-one were treated in hospital—where the death occurred—and twenty were treated at home.

The circumstances of the outbreak seem to us worthy of being put on record, although the actual source of the infection of the milk was not discovered. The teat eruption on certain of the cows that supplied the milk was probably no more than a coincidence. It is, however, necessary to state that our investigation did not begin till April 30th, by which time the most acute period of the outbreak was past.

Course of the Outbreak.

The outbreak began in the second week of April, and continued through May and June, the last case having been notified on June 23rd. The majority of the cases sickened in April, from the 10th onwards, no less than fifty-one having sickened between that date and the last day of the month. The later cases were largely secondary to former cases in the same household, and need not be considered from the ætiological point of view.

Cause of the Outbreak.

The outbreak was sudden in its onset, and stood by itself, there having been no cases of scarlet fever notified in the town between January 10th and April 14th of this year. But during last autumn and winter (between September 9th, 1909, and January 10th, 1910) fifty-one cases of scarlet fever had been notified to the medical officer of health.

Detailing the sickenings in April, we find that seven cases sickened between the 10th and 14th, thirty-three between the 15th and

22nd, and eleven between the 23rd and 30th April, or in all fifty-one sickenings in the last three weeks of the month. The suddenness of the onset of the disease, and its occurrence in more than one member of several families about the same time, suggested milk as the probable cause of the outbreak; and on investigating the milk supply of the first fifty cases it was found that twenty-eight had obtained all their milk from dairyman "A," while eighteen had had milk both from dairyman "A" and from dairyman "B." Only two had no milk from dairyman "A." The age incidence of the first forty-nine cases, of which the ages are known, was the following:—

2 were under 5 years of age,
14 were above 5 and under 10 years,
13 were above 10 and under 15 years, while
20 were above 15, 13 of them being over 18 years of age. Of the 20 cases over 15, 12 were females and 8 males.

The age incidence of the disease showed that it was largely prevalent among those over school age, while the number affected at about the same time was also against the idea of case to case infection.

The facts as to the milk supply of the cases have already been drawn attention to, and the conclusion drawn was that the milk from dairyman "A" was associated with the spread of the disease, and probably caused it. This was strengthened by the local distribution of the cases in the burgh, which coincided with the area served by the dairyman's cart, as was well brought out by a casual remark from the local sanitary inspector that the disease did not go higher up the hill than a certain house,

which was the last at which "A's" cart left milk.

Up till May 29th four cases of scarlet fever had occurred among the dairyman's county customers, who numbered about twenty-one. Three cases sickened in April, viz.: on 16th, 24th and 25th. In the case of the first, all the milk came from dairyman "A." In the other three, part of their milk supply was from that source. The first two cases (females) were aged seventeen and sixteen years respectively, and were in the burgh daily; the third, aged six years, was a boy in school in the burgh.

Description of Dairy and of the Milk Business.

The dairy premises are situated a little outside the burgh and consist of two cowsheds, in which there were twelve and four cows respectively on April 30th. The cowsheds are constructed of wood, and were airy, clean and light when visited. The animals looked well cared for, and seemed to be groomed regularly.

The milk-house, at some distance from the byres and also constructed of wood, was clean; it did not communicate with the dwelling-house. The facilities for the cleansing and washing of milk dishes were satisfactory and were situated in a separate outhouse. At some distance from the byres was a piggery, which was clean and well kept when seen.

The water supply was obtained from a dip well, built up above the level of the ground and covered. We were informed that it had been clay-puddled around its entire extent for a distance of several feet from the surface some years ago. The dungstead was at a lower level,

and at some distance away. Manure was not allowed to lie on the ground near the well: on several visits to the ground no contamination of the surface in the vicinity of the cowsheds or of the well was observed.

The dwelling-house, the under-floor of which is used partly as a wayside inn, is quite separate from the cowsheds or any of the outhouses used in the milk business. It was tidy and in all respects satisfactory on the several occasions on which it was visited. The dairy people impressed us favourably, being intelligent and cleanly.

They comprised the following: —

1. The dairyman's mother.
2. The dairyman.
3. His sister.
4. The dairymaid, an employee.
5. An old man, who prepared food for the cattle, broke up oil-cake, etc.

Of the above, the mother took no part in the dairy business; nor did the old man who prepared the cows' food do any milking.

The dairyman and the maid alone attended to the cattle in the byres, and milked the cows. The dairyman's sister did not milk, but cleaned the milk dishes and helped generally in the business. Everyone in the dairy was examined by the county medical officer of health and by one of us. No evidence of scarlet fever was found in anyone; nor was there any suspicious history, such as recent sore throat, in the case of any of the people at the dairy. No one showed evidence of sores on the hands. The dairyman and his sister had had scarlet fever as children, their mother said.

We were informed that there had been no

outside assistance in the dairy business for a period of fully six months, nor had milk been obtained from any outside source to supplement the dairyman's supply for about a similar period.

The dairy people had not been out of their own house even for a day for several months, and they had had no visitors staying with them. The business they carry on, in addition to the dairy, is not an hotel, only a wayside inn for the convenience of passers-by.

Lastly, the medical officer of the county stated that no connection could be traced between the cases of scarlet fever of which he knew the existence in two situations in the county and the dairy concerned.

Method of Distribution of Milk.

The dairyman distributed his milk to customers in the following manner. Three boy messengers came out to the dairy every morning and evening from the town. They each received a number of cans with milk, which they carried first to customers in the outskirts of, and then to others in, the town. When the cans were empty they were refilled from the cart, the dairyman having by this time started on his rounds himself. The dairyman himself drove to the town, and on his cart were several "butts" and some cans with milk, as well as measures. At the mid-day delivery there were no messengers, the dairyman doing all the business himself when the boys were at school. The milk cans and measures were all the property of the dairyman, and were not left in customers' houses, the milk being merely measured into customers' own vessels. All dishes, etc., were washed and

scalded at the dairy, where there is a special boiler for the purpose, after each delivery.

The medical officer of health of the burgh examined all the milk carriers at an early stage of the outbreak and was satisfied that none of them had the disease, and also that the disease was absent in all their families. There was a rumour that the father of one boy was suffering from scarlet fever, or from illness of a suspicious nature. A visit to the house showed that the rumour was unfounded. As a matter of fact, the man had had scarlet fever as a young lad, but not since, nor was any member of his family affected.

It will be remembered that a second dairy in the town supplied part of their milk to eighteen of the first fifty-one cases. The burgh medical officer of health visited here also and satisfied himself of the absence of any evidence of scarlet fever among those engaged in the dairy business.

Although the association of a particular milk supply with the cases was so marked as to justify the conclusion that the particular milk was spreading the disease, no evidence of scarlet fever was found in anyone engaged in any part of the milk business. But, as already pointed out, the inquiry was not begun till the acute part of the outbreak was over. One of us visited the cases in hospital, along with the doctor in attendance. They had all the characteristics of cases of scarlet fever.

Stopping of the Milk from the Dairy.

The milk from the dairy was not stopped till April 30th, and its sale was resumed on May 9th, without giving rise to any undue

spread of the disease, such as had existed before April 30th.

Eruption on Cows' Teats in the Dairy the Milk of which was Associated with the Spread of Scarlet Fever.

The existence of an eruption on several of the cows' teats in the dairy the milk of which was associated with the outbreak was made known to the Board, and they arranged that Dr. McGowan, bacteriologist in the Royal College of Physicians' Laboratory, should accompany their medical inspector to the dairy on April 30th, and that he should report the result of his bacteriological investigations to them. The description in detail of the conditions found in various cows is left to Dr. McGowan—a general description of the condition, and the question of the probable date of onset of the eruption, being the only matters dealt with here. The eruption on the teats affected several of the cows in the dairy, and having begun in one or possibly two of the animals was communicated to others, presumably by the milkers' hands. From what the dairymaid said, it seemed that the eruption began as a sort of papule, and passed through the stages of vesication and pustulation before drying up in the form of a crust.

The date of onset is somewhat difficult to fix; in fact, a definite date could not be put on it by the dairy people, although they stated that no cow was affected before about April 18th. But it was admitted that *several cows* were affected on April 21st; while a recently calved cow came to the byre on April 9th, and though not affected at that date, this animal

developed the eruption later. Dairy people are not very observant as a rule, and they tend to regard any pathological condition of their animals as an aspersion on themselves, particularly when, as in this case, the milk they sell is suspected of spreading disease. The statement that the teat eruption did not begin before about April 18th is therefore one the accuracy of which it is permissible to doubt. But there is no evidence that the eruption existed on any cow's teat about the end of the first week of April.

It cannot therefore be contended that the connection between the teat eruption and the outbreak of scarlet fever among people taking the milk was more than an accidental one.

In this connection it is interesting to be able to state that no similar condition existed in the cows of any other dairyman in the town. A visit paid with the veterinary surgeon to all the dairy premises, and an examination of the animals therein, proved that they were not similarly affected, and that the rumour to this effect was unfounded.

That the milk was spreading the disease cannot be doubted from the facts submitted. Personal infection from case to case does not satisfactorily explain the circumstance that thirty-three people, all of whom partook of the milk, sickened of scarlet fever in the course of a single week in a small town of about 2,000 inhabitants. But a human source of infection of the milk was not discovered, and though the teat eruption was probably present in a number of the cows during the height of the outbreak, there is no evidence that it was the

cause of the scarlet fever, nor that in point of time it could have caused the outbreak.

One has therefore to admit that the source of infection of the milk was not discovered.

From our knowledge of "carriers" of infection in diphtheria it may be surmized that a similar condition is sometimes found in scarlet fever; but in the absence of definite knowledge of the causal organism of the latter disease the existence of carrier cases in scarlet fever cannot be demonstrated.

Bacteriological Report by Dr. McGowan.

On April 30th, along with Dr. Dittmar, I paid a visit to the dairy. There were sixteen cows in it, twelve being in one byre and four in another. I examined each cow individually, with regard to the condition of the teats, udder, and skin. The general state of health of the animals and their temperatures were also noted.

On the teats, and on the udders at the base of the teats, in seven out of twelve cows in the larger byre I found ulcers. These ulcers were of small size, generally not bigger than a threepenny piece. Where they occurred in the path of cracks in the skin they were slightly bigger. They were very shallow. Their edges were covered over with thin, dry epidermic scales from the surrounding skin, with which they were practically flush. There was no surrounding areola of inflammation, nor was the site of the ulcer swollen. The surface of the ulcer, except where it had been recently torn off, consisted of a thin, dry, brownish scab. Where this surface had been rubbed off, as by milking, there exuded a small amount of clear serum. The ulcers were not tender, for the cows allowed the teats to be handled with

the utmost freedom, and they had also been regularly milked without difficulty. The teats themselves were not swollen.

The number of ulcers on the teats varied considerably. Usually the number was about three or four per teat, though in one case thirteen ulcers were counted on one teat. In some cases only one teat of a cow was affected. The ulcers sometimes occurred on the udder, but there they were confined to the udder at the base of a teat.

The above was the appearance of the ulcers at the time of my examination, but it is possible that they had been modified somewhat in appearance by the application of a lotion which had been used. As regards the earlier stages of the eruption, it is difficult to say what the appearances were. The owner of the cows told me that the eruption began as a small pimple, enlarged to the size of a split pea, became yellowish and then formed an ulcer. Sometimes the condition aborted without an ulcer forming. The formation of the ulcer, when it occurred, took place within three days. I did not myself see the eruption in the papular stage and I only observed the pustular stage in one instance. The pustule was about the size of a split pea, was very slightly raised above the surrounding surface, was without umbilication and was not surrounded by any inflammatory zone—in fact, the surrounding tissue appeared perfectly healthy. The pustule was yellow coloured, was enclosed by thick skin, and when punctured a small quantity of viscid tenacious pus was obtained from it.

As regards the cows in the second byre, four in number, they were affected to a much less

degree than those in the larger byre. Only two or three ulcers were found on the four animals and no pustules were observed.

In none of the animals in either byre did I observe any falling off of the hair or any eruption elsewhere than on the teats and udders. The cows were all with one exception in excellent condition and took their food well. The cow noted as an exception was rather thin. No diminution in the quantity of milk obtained from individual cows had been noted during the course of the disease. The temperatures of all the cows were within the normal limits.

Eleven days after the first visit I paid a second visit to the byre. Apart from the fact that a new cow had been received into the byre that day and that a cow had been transferred from the larger byre into the smaller one, the contents of the byres were the same as on the last visit. But owing to local treatment of the disease the ulcers were practically all healed, and it was with difficulty that any material was obtained for inoculation of tubes.

On both occasions agar, broth and litmus milk-tubes were inoculated on the spot with milk from ulcerated teats of the cows and with material from the ulcers. Similar tubes were also inoculated with the contents of the one pustule that was found. Milk was also received into sterile bottles from several of the affected cows to be conveyed to the laboratory. There plates were made for isolation of the organisms present, and small rabbits were inoculated intraperitoneally with 2 c.c. of the milk. Some scab material also was removed in a sterile test tube, and plates were made from an emulsion of

it on arrival at the laboratory. Part of this material was kept for inoculating a calf.

As regards the rabbits which were inoculated intraperitoneally with 2 c.c. of the milk, none of them showed any signs of illness.

Streptococci were obtained both from the milk and from the ulcers. They were non-pathogenic to rabbits and mice, grew in short chains in broth, were non-hæmolytic, and grew on gelatine at 20 deg. C. They clotted milk, and produced acid in lactose and saccharose. They would thus appear to belong to the salivarius group of streptococci. No streptococci were obtained from the cultures made from the pustule, nor were any streptococci observed in films made from its pus.

The organisms other than streptococci which appeared on the agar tubes, plates, etc., were isolated in pure culture, but no attempt was made to identify them. They, along with the streptococci and the scab material, were used to make inoculations into the skin of a calf to see if the eruption on the cows' teats could be reproduced in the calf. A four-days-old calf was obtained, and the hair shaved clean off along the whole length of its back and down its sides. Circles were then painted on with eosin along the shaved portion to mark the inoculations. The inoculation was made by taking a large quantity of the infecting material—culture, etc.—putting it as the centre of the eosin circle, and pricking it in with a sterile sewing-needle. Most of the inoculations caused no reaction, and the few which did gave none of the appearances of the eruptions on the cows' teats.

Nothing definite is known concerning the

causal organism of scarlet fever. Streptococci have been isolated by some investigators from cases of scarlet fever, and have been thought to be the cause of the disease. Klein, too, isolated streptococci from the lesions in the cows whose milk was associated with the scarlet fever outbreaks at Hendon and Glasgow, and arrived at the same conclusion. Streptococci, however, can be obtained from the milk and udders of cows removed from any suspicion of causing scarlet fever. In this epidemic streptococci were obtained indifferently from the milk of cows with and without the eruption on their teats. They were obtained, too, from the surface of the ulcers, but it is to be noted that, in the one pustule that was present, streptococci were not found either microscopically or culturally.

As regards the ætiological connection of the eruption with the outbreak of scarlet fever, it is to be noted that the epidemic was well advanced before the eruption was noticed on the cows' teats. As regards the nature of the eruption itself, from its general appearance as described above, and from the fact that it was not infectious to the attendants on the cows, it will be seen that it corresponds very closely to the condition known among veterinarians as "pseudo-vaccinia" or "false cow-pox." This is a very common infectious disease among cows, and usually occurs without any associated epidemic of scarlet fever among persons consuming the milk.

ON THE RELATIONS OF THE LYMPHATICS OF THE SPINAL CORD.[1]

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(Plates XXIII.–XXVII.)

It is becoming more and more evident that an accurate knowledge of the lymphatic system of the spinal cord (and the same holds equally true with regard to the brain) is essential for the proper interpretation of the pathological processes which take place within it. Much has hitherto been attributed to the blood vessels which there is increasing reason to think is in reality to be associated rather with the lymphatic system. The latter is admitted to be the main channel for the removal of waste products and products of degeneration of the constituent elements of the central nervous system, more especially of the myelin. Attention is now being more and more drawn to the effects of disorders of the lymphatic circulation—such as stasis and dilatation of the spaces—upon the nervous elements, but little definite information is as yet available. Arndt (1870[1]) was one of the first to recognise the influence of a hyperlymphosis or lymphostasis upon the specific elements of the central nervous system, and since his time various important contributions have been made to this subject, some of them quite recently. There is an accumulating body of evidence that the lymphatics play a part probably not subordinate to that of the blood vessels as channels by which infective agents (toxins and organisms) are conveyed to the cord or distributed within it. Among other papers we may refer to the work of Meyer on tetanus, of Orr and Rows (1907[2]) on the flow of the lymph stream in the spinal roots of the cord, and to the classical work of Wickman on poliomyelitis.

Lymph Channels.

It is generally admitted that the brain and spinal cord do not possess lymph vessels closed in by walls of their own. The lymph circulates in spaces around the blood vessels, and these spaces communicate with the lymph sacs

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in the membranes. Our present knowledge of these channels is largely due to the early researches of His (1865 [5]), Robin, and Obersteiner (1900 [7]), and later of Axel Key and Retzius. In the text-books of Obersteiner and Schmaus-Sacki (1901 [12]) we find it stated that within the spinal canal from without inwards are found: the epidural space, filled with fat and venous plexuses; the dura covered on both sides with endothelium, and composed of dense connective tissue in which are numerous narrow lymph spaces communicating with the subdural space; the subdural space, normally a capillary space; the arachnoid, a non-vascular membrane covered on both surfaces with endothelium; the sub-arachnoid space, in which circulates the cerebro-spinal fluid, and crossing which are numerous connective-tissue threads dividing it into larger and smaller lymph sacs (this space communicates with the lymph spaces in the pia and also with the lymph spaces within the perineural sheaths); the pia, in which are found the blood vessels which penetrate the cord, also numerous lymph spaces which communicate with the sub-arachnoid space; the epispinal space or space of His, a fissured space often permeated by radial fibres; dense glia forming the cortical surface of the cord (Lenhossék's membrane or *membrana limitans gliæ superficialis*).

In early development the central nervous system has no blood vessels, and these first enter as buds from the pia into the up till now solely ectodermal tissue, carrying before them the *membrana limitans superficialis* as a *membrana limitans perivascularis*. With the buds there also enters loose connective tissue from the pia, which plays the rôle of adventitia, and the spaces of which are continuous with the spaces of the pia and through them with the sub-arachnoid space. Therefore, between the adventitia and the *membrana limitans perivascularis* we have a space—the perivascular space of His—continuous with the epispinal space of His, and a continuation of the sub-arachnoid space in the loose tissue of the adventitia—the adventitial lymph space or Virchow-Robin space. These two lymph spaces are said to exist everywhere independently of each other around the vessels of the central nervous system, one within the adventitia (intravascularis), and one outside the adventitia (extra- or perivascularis). Where the adventitia is absent or hardly perceptible, as in the finest capillaries, the two spaces blend into one, and there is only one space between the limiting membrane and the capillary endothelium. Further, each ganglion cell is surrounded by a wide space—the space of Obersteiner—through which it sends its processes and within which two or three lymphoid cells are frequently found. Obersteiner's diagram, copied into most text-books, has familiarised us with his statement that there is a direct transition of the perivascular space into the pericellular space, and that the ganglion cells are thus embedded in the perivascular lymph system.

The tissue lymph spaces discharge everywhere into the perivascular space, either through the interstices of the glia tissue, or by the protoplasmic processes of the glia reticulum, and the lymph flows out in the direction of the periphery of the cord. A lymph flow outwards is also assumed in the adventitial lymph sheaths.

The lymph channels of the anterior and posterior roots are also in communication with the lymph system. Orr and Rows (1907 [9]) have pointed out the evidence of a continuous flow of lymph upwards along the peripheral nerves and spinal roots. Their researches prove that the main current lies at the periphery of the nerve bundles in the inner meshes or lymph spaces of the fibrous perineural sheaths. The current, as it reaches the cord, passes partly to the lymph spaces of the pia-arachnoid, but mainly along the nerve roots into the substance of the cord.

According to this account, therefore, we have two independent lymph spaces, namely, the inner, known as the Virchow-Robin or

adventitial space, continuous with the sub-arachnoid space, and the outer, known as the perivascular space of His, continuous on the one hand with the epispinal space of His, and on the other hand with the pericellular space of Obersteiner. Regarding the adventitial lymph space all is clear and definite; regarding the perivascular and pericellular lymph spaces, however, many false views have been promulgated, and these spaces are often confounded with another space which is regarded by many observers emphatically as an artefact.

In transverse sections of the cord fixed in formalin or chromic salts and hardened in alcohol, obvious clefts are found around the vessels and cells. His (1865 [4]) stated that these clefts were smooth, and that the outer wall was formed of a condensed layer of glia substance covered with endothelial cells. His further succeeded in completely filling up the perivascular and pericellular spaces by injecting from the epispinal space. Obersteiner (1900 [7]) found, in the presence of small cells, indistinguishable morphologically from lymphoid cells, within the pericellular spaces, a proof of their being lymph spaces. Friedmann ([3]) also came to the conclusion that the perivascular and pericellular spaces were preformed and lined by endothelium analogous to lymphatic endothelium.

More recent investigations have proved that the occurrence of the spaces of His and Obersteiner can be avoided, and that they are due to shrinkage through the methods of fixation. A careful consideration of the clefts shows that they rarely possess the smooth walls ascribed to them by His, and that where such is the case, the endothelial lining of His and Friedmann is undoubtedly a layer of glia cells which have so folded themselves round a space through the tissue retraction. More usually, fine threads of glia fibrils connect the adventitia of the vessel or membrane of the ganglion cell to the ragged outer wall of the space (Plate XXIV. Figs. 5 and 6). Obersteiner's picture of "a rose-branch beset with thorns" can thus be traced to fine glia fibrils which have remained existent between the vessel and the retracted tissue (Plate XXIV. Fig. 5). Obersteiner admits that the perivascular and pericellular spaces may increase in size through tissue retraction, but maintains that the normal existence of such spaces is proved by the presence of the free round cells already mentioned.

Many advocates of the perivascular space admit the presence of these fine threads connecting the outer wall with the adventitia of the vessel and with the ganglion cell, and amongst these Schroeder (1908 [13]) maintains that the true perivascular space of His is still within the *membrana limitans perivascularis*, which remains adherent to the ganglion cell or adventitia, and that the shrinkage space is external to this limiting membrane.

The usual treatment with alcohol in the process of hardening gives rise to considerable shrinkage on account of the varying consistence of

the individual elements of the tissue, and so we get rupture at certain parts. One of the parts of predilection is in the neighbourhood of the vessels. The tear here is directly external to the *membrana limitans perivascularis*, and as this is a condensed layer it remains adherent to the vessel adventitia, while the glia tissue outside retracts. Such artificial shrinkage spaces are constantly found in preparations, and the vessel as a rule lies somewhere on the wall of the space—not in the middle (Plate XXIII. Fig. 3). The space is traversed by individual small fibres radiating from the perivascular glial ring round the vessel. In œdema of the cord and in arterio-sclerosis we find that these shrinkage spaces are specially large.

Amongst those who have most strenuously opposed the teaching regarding the perivascular and pericellular lymph spaces is Nissl. Nissl has followed Degenkolb and Weigert in looking upon the adventitia as a biological limiting layer, separating mesodermal from ectodermal constituents of the nervous system. He finds in this biological limiting layer the key to the understanding of the histo-pathology of the nervous system. Nissl (1903 [6]) claims that there is no penetration of mesodermal elements into the nervous substance so long as this limiting layer remains intact, and that were the perivascular and pericellular spaces really lymph channels with endothelial nuclei in their walls, the parenchyma of the nerve tissue would be over-run with lymphoid (mesodermal) elements. To grant the existence of preformed perivascular and pericellular spaces would be, he asserts, to mistake and underestimate the part played in the histo-pathology of the cord by the non-nervous cells of ectodermal origin. In inflammatory conditions in the cord and in slow degenerations the reparatory processes within and without this limiting layer are altogether independent of each other, but if ectodermal or mesodermal elements are so injured that they could no longer form checks to the growth of uninjured elements, then the destroyed biological balance would be restored by progressive processes on the part of constituents of either origin. These views are too theoretical to be of service in the solution of the problem.

Artificial Injection of the Lymphatics.

His and Obersteiner injected the perivascular and pericellular spaces from the epispinal space, but there are many objections to such a method, the chief being that the very delicate nature of the nerve tissue causes it to give way at the points of least resistance under the pressure. Key and Retzius found it impossible to inject the perivascular and pericellular spaces from the sub-arachnoid space, from which they inferred that these spaces are not in communication with the large lymph spaces of the central nervous system.

Many observers see in the epispinal space a result of tissue retraction. This would indeed almost follow from the absence of the perivascular space, for if the adventitia is closely applied to the *membrana limitans perivascularis*, then the *pia intima*, which is carried in as the outermost layer of the adventitia, must be closely applied to the *membrana limitans superficialis* without any epispinal space. In

tissues fixed in Zenker's solution, and very gradually carried through the different hardening fluids, we find that this *pia intima* is closely adherent to the surface of the cord and that no perivascular or pericellular spaces are present. Ranke (1908 [10]) has also found this to be the case in frozen sections from fresh material and in tissues fixed in Cox's solution.

It will be seen from what has just been said that artificial injection of the lymph paths of the cord gives unreliable results. Fortunately, however, there is a natural process of injection of the lymphatics which can be depended upon as being absolutely selective, and which is therefore eminently suitable for the study of the lymph paths. This process occurs in every case in which a slow degeneration of the myelin is taking place, and it is seen specially well in cases of long-continued compression of the cord, or in the sub-acute combined degenerations such as are so often seen in pernicious anæmia. Here we get the most marked distension of the *adventitial lymph spaces* by cell elements filled with fat—the so-called compound granular cells. The course of all the vessels, arterial, venous, and capillary, can be traced by the dense layer of these cells which surrounds the vessels. *In every case it is significant that the cells have pushed apart the connective-tissue fibrils of the adventitia and that an outer layer of adventitia*, pink with Van Gieson's stain, *bounds the dilated lymph spaces and is closely applied to the glial membrane, leaving no perivascular space.*

Van Gieson's method of staining is particularly well adapted to demonstrate the boundary of the lymphatic space and its relation to the surrounding glial tissue, as it stains the connective tissue a more or less deep pink colour, and the glial tissue an orange-yellow colour. As will be seen from Plate XXIII. Figs. 1 and 2, the space surrounding the small arterioles, venules, and capillaries is bounded by the pink line of connective tissue which represents the outer layer of the adventitial lymphatic sheath.

Compound Granular Cells.

When the degeneration of myelin has reached a certain stage, then resorption of the degenerated material sets in by means of granular cells. The space occupied by the degenerated nerve fibres becomes replaced by granular cells. These may remain a shorter or longer time, but finally they reach the lymphatic spaces in the adventitia, and are thence carried to the inner layers of the pia. (Nissl named these compound granular cells *Gitterzellen*, or lattice cells, because he considers that the lattice structure of the protoplasm—found after the fat is extracted—is the chief characteristic of these elements (Plate XXIII. Figs. 1 and 2).) The fat may be absorbed by the cell in the form of clumps of degenerating myelin, or in a dissolved form as saponified fat and, by synthesis within the cell, be transformed

again into neutral fat when it appears as fine droplets (Plate XXV. Figs. 9 and 10). Simultaneous with the work of phagocytosis of the fat the solution of the degenerated myelin takes place. This process is helped by the lymph stasis through its power of distending and dissolving the nerve fibre. The fluid components may become directly absorbed by the cells as just stated, or be absorbed directly into the lymph spaces; and one finds in the lymph sheaths, in addition to granular cells, free granular, fatty detritus. The granular cells at first fill up the space of the degenerated fibre, and on longitudinal sections of the cord are seen in rows in the tubular space originally occupied by the nerve fibres. On transverse section they appear as large, rounded cells with faintly staining, finely vacuolated protoplasm (Plate XXIII. Figs. 1 and 2). As the granular cells become laden with fat, the adventitial lymph spaces are opened up, and the peripherally streaming lymph, acting as a suction pump, draws the cells into the spaces. The number of the compound granular cells varies with the duration of the process and the nature of the degeneration. When all the decayed material has been absorbed by the cells, and these have been drawn into the lymph spaces of the adventitia, a dense glia tissue, with narrow tissue spaces, takes the place of the degenerated fibres and compound granular cells. The latter may still be found isolated in the tissue and immediately around the vessels. Through a further resorption of the fat from the cell—the fat may be absorbed into the lumen of the vessel (see Plate XXV. Fig. 9) or into the lymph spaces and become free—the granular cell undergoes atrophy, and vessels may be found in densely sclerosed areas where the granular cells in the adventitial lymph spaces have given up their fat. Such cells remain in the adventitia as "ghost cells," with shrunken nuclei and the remains of cell body and membrane, to increase the nuclear content of the adventitia. Through this process comparatively few of the large numbers of compound granular cells reach the surface of the cord, and the course of the vessels always shows a marked diminution of the dilated adventitial spaces near the periphery of the cord (Plates XXVI. and XXVII. Figs. 11 and 13). The cells as they reach the pia spread into the spaces of its inner layers —the spaces of the adventitia being continuous with the pial lymph spaces. It has been stated that no perivascular space can be recognised in transverse sections of the cord. The cells fill out the spaces of the adventitia and separate its fibrils to so great an extent that the outermost layer of the adventitia is closely pressed against the *membrana limitans perivascularis*. Likewise, on the surface of the cord *no epispinal space can be recognised*, for the innermost layer of the pia, stained pink by Van Gieson's stain, can be found closely applied to the *membrana limitans superficialis* and distinctly within the layer of granular cells, filling the lymph spaces of the *pia intima*. Even in Marchi preparations it can be distinctly noted that the compound granular cells are spreading in the inner pial layers, not in an epispinal space. Such

natural injections of the lymph spaces therefore lend no support to the view of the existence of perivascular and epispinal spaces. It should also be noticed, as a further important evidence of the non-lymphatic character of the so-called space of His, that in all the preparations in which there was natural injection of the lymphatics, where the process of fixation and hardening had produced a shrinkage space between the vessel and the surrounding nervous tissue, this space was invariably devoid of compound granular corpuscles. It is inconceivable that this could have been the case had His's space been really a lymphatic space.

With regard to the pericellular spaces, there is no corresponding natural injection by compound granular corpuscles. It is quite true that they may have in their neighbourhood one or more lymphoid corpuscles when the cells are in a state of degeneration, but it is not clear that these are lying in lymphatic spaces. There is no indication of any endothelial lining to the space outside the cell, and if the tissue has been carefully hardened by Zenker's method, no fissure will be seen. If the process of fixation has been imperfect, a space may appear either round the cell and its processes or in relation to part of it, but in such cases careful examination will show, in many instances, that the process of fissure formation has begun by rupture of the glial tissue immediately surrounding the nerve cell, and that a portion of its tissue has been left adherent to the nerve cell, with ragged threads projecting either into or across the fissure (Plate XXIV. Fig. 6). In our opinion the process of pericellular fissure formation is exactly analogous to that of the perivascular one. There is no more a space of Obersteiner than there is a space of His. Both are artefacts due to imperfect fixation of tissue, aided frequently, no doubt, by pathological changes which render the glial tissue less resistant. In both instances the rupture is through the glial tissue, part of which remains attached either to the vessel or the cell, as the case may be. We are of opinion that there is but one set of lymphatic vessels, namely, the adventitial, which, obtaining its lymph supply either from the vessels or absorbing it from the nerve tissue, conveys it into the deep layers of the pia.

An examination of various cases of degeneration of the white matter shows that the main course of the lymphatic current goes towards the periphery of the cord. In the case of pernicious anæmia, which was the subject of most of the illustrations of this paper, sections stained by Marchi's method showed no evidence that the compound granular corpuscles were making their way either towards the central canal or the grey matter of the cord. The latter fact appears to us to be an additional argument in favour of the intimate relationship of lymphatic and blood vessel, as the vessels of the white and grey matter of the cord are almost entirely independent of each other. Therefore, in an involvement of the distribution of the

vessels of the white matter, the lymphatics of the other area would remain unaltered.

In conclusion, briefly: Within the spinal cord there is no lymphatic epispinal space of His; there is no perivascular space of His; there is no pericellular space of Obersteiner. The lymphatic channels, as far as we know, follow the adventitia of the capillaries, veins, and arterioles towards the surface of the cord, where they enter into the deep layer of the pia mater, through which they probably communicate with the sub-arachnoid space. The lymphatic path has in the main an outward direction, but there is no doubt that it permits of a current inwards or of an invasion by cellular elements, micro-organisms and toxic substances.

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DESCRIPTION OF PLATES XXIII.–XXVII.

Plate XXIII.

Fig. 1.—Transverse section of three small vessels; distension of their adventitial sheaths, with compound granular corpuscles. Zenker fixation. Van Gieson's stain. (× 400.)

Three capillaries are seen surrounded by a closely compacted number of compound granular corpuscles. These are enclosed by the outer layer, stained pink, of the connective-tissue wall of the adventitia. No perivascular space is seen anywhere. Within the adventitia connective-tissue cells are seen between the granular corpuscles.

Fig. 2.—Minute capillary transversely divided: adventitial sheath distended by a single ring of compound granular corpuscles. Note pink outline of adventitia immediately adjoining glia tissue, leaving no evidence of perivascular space.

Note the glia cells and compound granular cells in the adjacent tissue. Van Gieson's stain. Zenker fixation. (× 500.)

Fig. 3.—Transverse section of a slightly larger capillary surrounded by ring of compound granular corpuscles enclosed within the adventitia, which is limited by a pink line of connective tissue. On the left of the vessel is seen a shrinkage space formed by the artificial separation of adventitia and glia. This is the space commonly described as the perivascular lymphatic. Van Gieson's stain. Zenker fixation. (× 500.)

Plate XXIV.

Fig. 4.—Small venule cut longitudinally, showing adventitial sheath distended with numerous nucleated elements. Note the complete absence of these from the space (shrinkage space) lying outside the vessel. Van Gieson's stain. Formalin fixation. (× 200.)

Fig. 5.—Longitudinal section of venule showing the formation of the perivascular shrinkage space. Tags and filaments of glial tissue are seen adhering to the outer layer of the adventitia of the vessel. The outer wall of the shrinkage space is similarly found. Van Gieson's stain. Formalin fixation. (× 320.)

Fig. 6.—Shows early stage of development of a pericellular shrinkage space. Fine fibrillæ of glia are seen passing over the space to the body and processes of the cell. Formalin fixation. (× 500.)

Fig. 7.—Section of pia mater, surface of cord, and entering vessel, showing the close adhesion of the inner layer of the pia to the condensed layer of the glia—membrana limitans superficialis—and the complete absence of any epispinal space. Van Gieson's stain. Zenker fixation. (× 320.)

Plate XXV.

Figs. 8–14.—Marchi sections from case of pernicious anæmia.

Fig. 8.—Longitudinal section of lateral column of cord. The compound granular corpuscles, stained black, form interrupted longitudinal lines leading towards, and surrounding, the capillaries. (× 40.)

Figs. 9 and 10, corresponding respectively to × and × × of Fig. 8, but magnified 250 and 200 times, show the extensive infiltration of the adventitia with the compound granular cells and free granules of fat.

PLATE XXVI.

FIG. 11.—Margin of cord with pia showing a peripheral vessel carrying compound granular corpuscles in its adventitial wall towards the deep layer of the pia mater, and within which they spread in both directions. Note the narrowing of the stream of corpuscles as the surface of the cord is approached. (× 70.)

FIG. 12.—Longitudinal section showing how the compound granular corpuscles approach and enter the adventitia of the vessels, and spread in the deep layers of the pia. (× 70.)

PLATE XXVII.

FIG. 13.—Transverse section of posterior columns of cord showing early degeneration in the outer zone, and more advanced change in the inner zone, where the black staining is restricted to the periphery of the vessel wall. The paler appearance of the inner zone is due to the compound granular corpuscles having been drained from the tissue along the adventitial lymphatics. (× 30.)

FIG. 14.—Transverse section of capillary showing a ring of compound granular corpuscles in its adventitia, and the approach of the cells in the tissue towards the vessel. (× 250.)

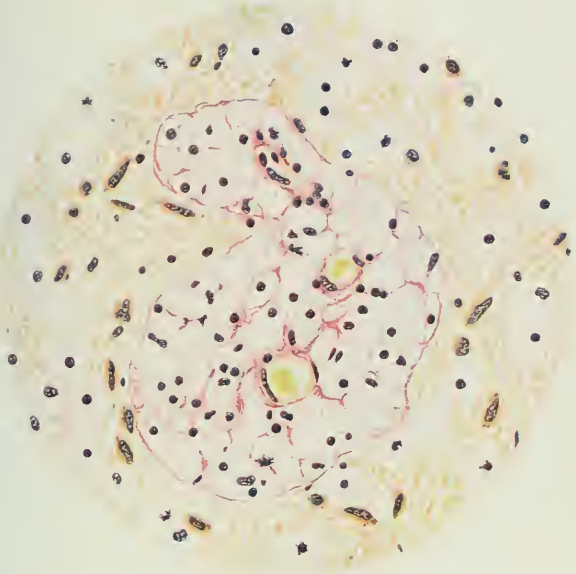


FIG. 1.

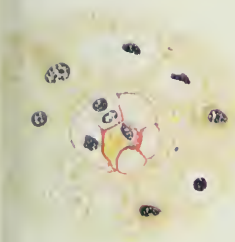


FIG. 2.

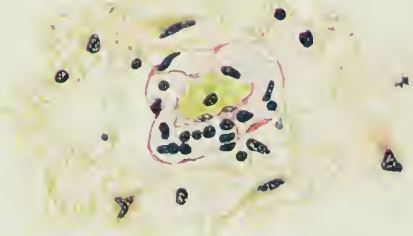


FIG. 3.

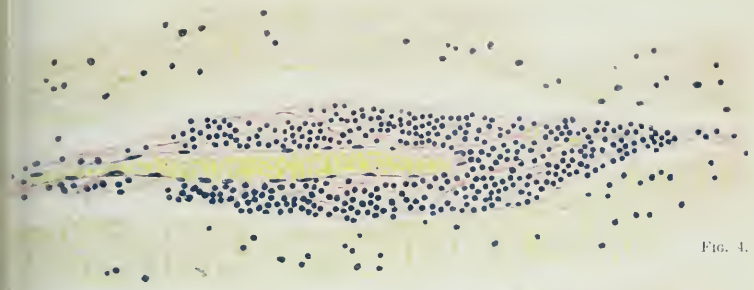


FIG. 4.

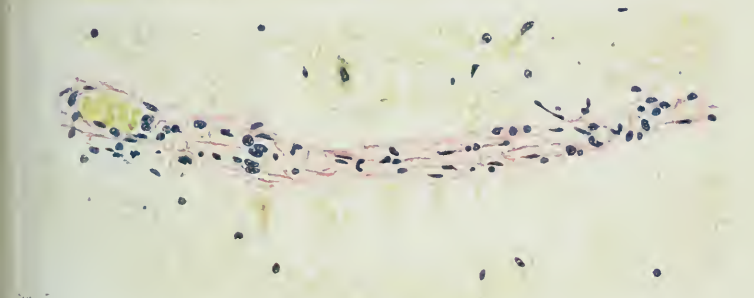


FIG. 5.

FIG. 6.

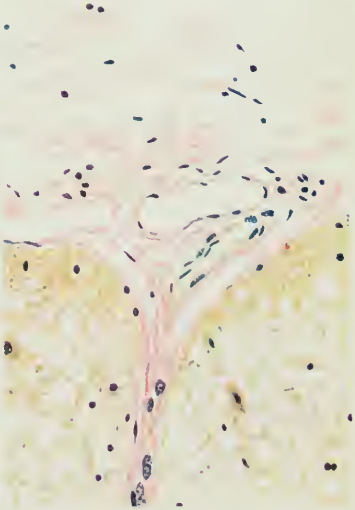


FIG. 7.

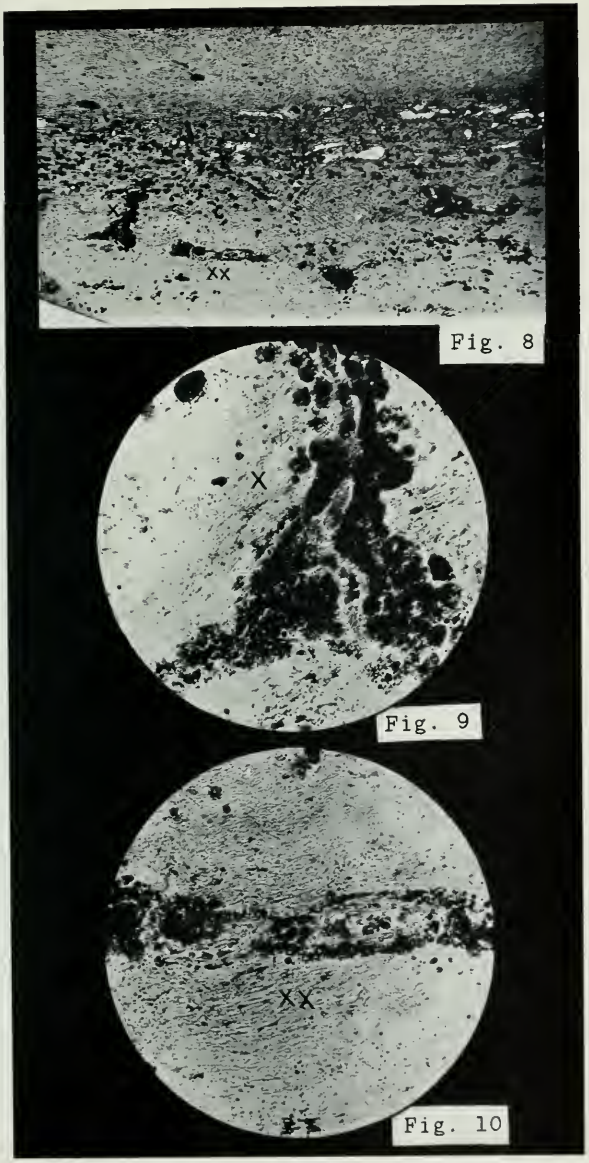
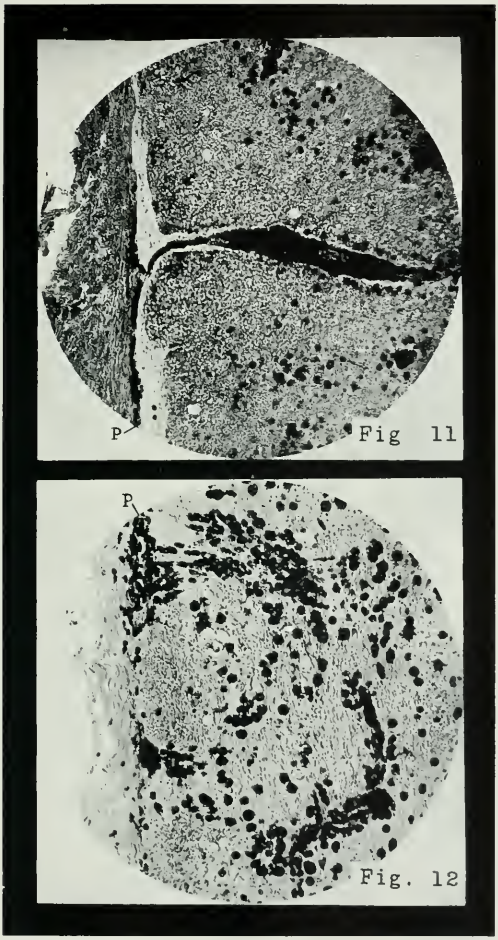


Fig. 8

Fig. 9

Fig. 10


P
Fig 11
P
Fig. 12

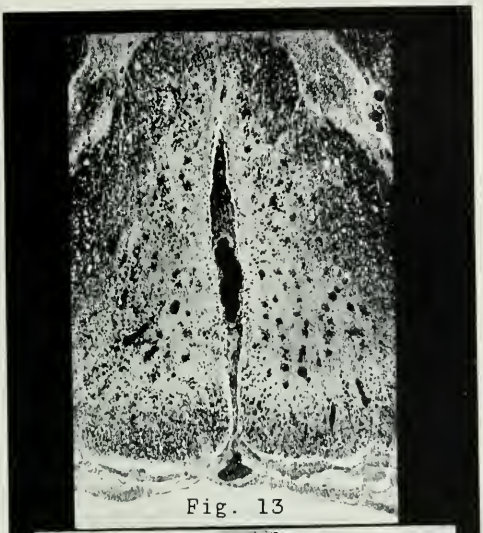


Fig. 13

Fig. 14

REPORT ON INVESTIGATIONS ON THE RELATIVE IMPORTANCE OF CERTAIN TYPES OF BODY CELLS IN DEFENCE AGAINST THE TUBERCLE BACILLUS, AND THE EFFECT OF TUBERCULIN UPON THEIR ACTIVITIES;

BY

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1911.

Appendix B., No. 6.

Report on Investigations on the Relative Importance of Certain Types of Body Cells in defence against the Tubercle Bacillus, and the effect of Tuberculin upon their activities; by James Miller, D.Sc., M.D., F.R.C.P.E.

It is by no means universally acknowledged that phagocytosis is a protective phenomenon. There are those who consider the taking up of foreign particles (including bacteria) by body cells as entirely due to physical forces which are not necessarily of a protective nature. There are, further, experiments carried out both "in vitro" and "in vivo" which go to show that bacilli which have been ingested by various phagocytic cells have not been thereby reduced in virulence when compared with the original organism. For example, in the case of the tubercle bacillus Baumgarten, working with leucocytes separated from the blood, and Bartel and Neumann, using aleurone exudate, failed to observe any difference in virulence between organisms before and after mixture and prolonged contact with phagocytic cells, although phagocytosis could be shown to have occurred. The last mentioned observers demonstrated that even when the cells and the bacilli came into contact within the peritoneal cavity and where phagocytosis could be proved to have occurred, samples removed from the peritoneum showed no loss of virulence for other animals. On the other hand the same observers after mixing bacilli with suspensions of lymph glands and incubating for varying periods, showed that after prolonged contact a loss of virulence was demonstrable. Also Opie injecting leucocytes, obtained by introducing turpentine into the peritoneal cavity, into tuberculous pleurae in animals, found that in animals so treated the disease progressed less rapidly.

Experimental results are therefore somewhat conflicting, a fact which merely indicates as might be inferred, *a priori*, that "in vitro" experiments do not represent conditions as they exist in the body, and that the difficulties to overcome in "in vivo" experiments are too great to afford the probability of obtaining reliable results.

Hence it seems desirable, with a view to throwing further light on the phenomenon of phagocytosis, to make a special study of the types of cells concerned in the taking up of tubercle bacilli both in spontaneous disease and under experimental conditions.

The subject naturally divides itself under two heads:—

1. The cells concerned in the taking up of tubercle bacilli as observed in tuberculosis of the human subject.
2. The cells concerned in this process under experimental conditions in animals.

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I.—Observations in Human Tuberculosis.

As is well known, the tubercle bacillus is comparatively seldom found in any great numbers in the lesions it produces, and when found it is usually in necrotic caseous material. The view of several authorities regarding the types of cells which ingest bacilli brought into contact with them is that these are chiefly of the connective tissue type. Baumgarten in an analysis of the literature on the subject up to date (1901) mentions no other type of cell. Orth (1901, 1906, 1907) admits that in certain instances the lymphocyte variety of leucocyte takes part in the inflammatory exudate in tuberculous pneumonia, but he does not mention anything about the bacilli being ingested by them. Councilman (1908) in an analysis of the tissue lesions produced by the tubercle bacillus states that the bacilli are found in part within the epithelioid and giant cells of the tubercle, in part between them. They are rarely found within polynuclear leucocytes and never within the lymphoid cells. He states, however, that occasionally tubercle bacilli may be found in great numbers within the polynuclear leucocytes.

The material investigated by me includes :—Several cases of acute tuberculous meningitis, acute tuberculous encephalitis, caseous broncho-pneumonia, diffuse caseous pneumonia, miliary tuberculosis, mesenteric tubercle, &c. The sections were stained with carbol- or anilin-fuchsin, being heated in the paraffin oven for thirty minutes ; they were then decolourised with 1 per cent. HCl in spirit and counterstained either with methylene blue or with Leishman's stain. I have also employed Much's method in a considerable number of cases and have compared the results with the appearances in the fuchsin-stained specimens, with a view to ascertaining more particularly if Much's granules are found in the interior of cells. This method is a modification of Gram's stain A full description of it will be found in an article by Berger (1909). Rosenblat (1909) considers that by this method a form different from that which is stained by the ordinary carbol fuchsin method can be demonstrated. It represents the nuclear material which remains after the disappearance of the acid-fast envelope. Wolff (1909) regards the structure thus stained as a latent form of the tubercle bacillus, one which may be found where the usual methods prove negative.

The results of this part of my investigation may be summarised as follows :—

Tubercle bacilli are rarely found in any considerable numbers in tuberculous lesions. When found they are chiefly extracellular in position, lying in necrotic material. But when isolated bacilli occur in small numbers at the margin of a caseous mass, these are found not infrequently within the branching type of cell usually called epithelioid. Occasionally they occur in large masses in caseous material with no stained cells in their neighbourhood.

They are occasionally found in large numbers either inside or in the neighbourhood of staining cells. For example, in acute meningitis the bacilli may sometimes be found in large numbers in the cells of the exudate in the pia-arachnoid, both in polymorphonuclear leucocytes and in the large mononuclear cells the nature of which is uncertain. In a case of acute encephalitis they were present in large numbers in the polymorphs and also in branching cells resembling neuroglia cells. Some of these latter which contained bacilli were multi-nucleated.

In cases of caseous broncho-pneumonia in children bacilli are often present in very large numbers in the zone of catarrhal consolidation in advance of the caseous change. Here they occur in the large mononuclear cells which are obviously endothelium thrown off from the alveolar wall, also in the smaller mononuclear cells which Orth regards as of the nature of leucocytes but which are certainly to some extent derived, by division, from the alveolar endothelium. Bacilli also occur in the polymorphonuclear leucocytes. These last are more numerous where the cells are breaking down. The bacilli become much less numerous when the completely caseous material is reached. Although frequently seen in close contact with the cells lining the bronchi I have never observed bacilli actually within these cells.

In acute broncho-pneumonic tubercle of the adult bacilli are, as a rule, much less frequently seen. When they are found it is chiefly in the centre of caseous masses away from cell elements or in the walls of cavities. In the latter position they may be observed inside polymorphs, sometimes in large numbers. Sometimes a mass of bacilli may be seen surrounded by polymorphs. The bacilli within these polymorphs show no evidence of loss of staining reaction; on the other hand the cells themselves frequently show evidence of degenerative changes, and sometimes masses of bacilli may be found with the faint indication of a cell body round them.

In tuberculous ulceration of the intestine in children bacilli are sometimes found in large numbers both in the branched mononuclear cells of tuberculous granulation tissue and in the polymorphs. In this case, as in the case of the phthisical cavity, the polymorphs may be there as the result of the presence of other germs.

In two cases of tubercle apparently primary in the intestine and with well marked lesions in the mesenteric glands where there were numerous giant cells, bacilli were found in large numbers in the epithelioid cells and also in the giant cells. Here also there was no indication by alteration of staining reaction that the bacilli were undergoing degenerative changes within the cells.

In sections stained with Much's method it did not appear to me that any more bacilli were demonstrated than by the older methods. The bacilli appeared more fragmented, but not more so within the cells than outside them. It is apparently only in caseous material that forms of the bacillus occur which can be brought out by this method, and which cannot be stained by the older methods.

To sum up. The tubercle bacilli are taken up by the polynuclear leucocytes in large numbers in certain cases even where there are no other germs present which might attract these cells. More usually the bacilli are found in cells which probably have a fixed tissue origin as, for example, the large catarrhal cells found in caseous broncho-pneumonia, also in the branched cells (often called epithelioid) found in tuberculous granulation tissue. They may also be found in neuroglia cells. They are not taken up by the columnar cells lining the bronchi. Where bacilli occur in masses and are not surrounded by caseous material the cells found in the immediate vicinity are usually chiefly polymorphonuclear leucocytes.

Bacilli are only very rarely to be found in giant cells in human tuberculosis. When they are found they occur at the same time in the epithelioid cells around. Their presence there appears to be

associated with a particular degree of virulence on the part of the organism and a particular state of resistance on the part of the individual. It does not necessarily mean a specially rapid rate of progress in the disease.

To put the results in another way, the cellular reaction to the tubercle bacillus under certain conditions differs in no way from that seen in the case of other organisms. As a rule, however, the tubercle bacillus being slow of multiplication, the reaction is of a chronic type in which connective tissue elements predominate.

II.—Experimental Observations.

Broden (1899) injected into the peritoneal cavity of dogs emulsions of bacilli, removed the exudate at intervals, killed the animals at stated periods and examined the tissues. Up to 24 hours after injection the chief cell present was the polymorphonuclear leucocyte. Many of these contained bacilli. After 24 hours, mononuclear forms began to appear; these Broden considers are the product of dividing endothelial cells. These cells, he found, frequently contained bacilli and steadily increased in number. No mention is made of mononuclear leucocytes or other forms of cells.

Beattie (1903) injected tubercle bacilli into the peritoneal cavity of guinea pigs, and subsequently examined the exudate and the tissues at stated intervals. The bacilli were found mainly in the mononucleated cells. A few were found in polymorphs. Beattie suggests that the polymorphs do not migrate into the peritoneal cavity so early after injections of pure cultures of tubercle as they do after the injection of other organisms. The mononucleated cells are present from the beginning and they take up the organisms just as they take up foreign bodies. Before the polymorphs have been "called out" the mononuclear cells have already ingested the bacilli.

Markl (1905) injected various strains, human and bovine, into the peritoneal cavity of guinea pigs. Fluid was withdrawn from the peritoneum from time to time by means of capillary pipettes and examined microscopically in stained films. After three hours the bacilli had been taken up in large numbers by the polymorphs. Many were still free; these later became swollen and lost their staining reaction. After two days the bacilli within the leucocytes showed loss of staining and granularity. After three days mononuclear forms began to appear in the exudate. Very few normal staining bacilli were found, the majority showing marked evidence of degeneration. After four days bacilli staining normally appeared in large numbers and were found within the polymorphs. Markl concludes that the polymorphs are the first line of defence. They take up the bacilli and act upon them so that they lose their characteristic staining reaction. In the process many of the cells are destroyed and in their place fresh polymorphs and mononuclear forms appear. The bacilli which are not taken up by the cells undergo a progressive degeneration.

Bartel and Neumann (1906) produced an artificial exudate in the peritoneal cavity of rabbits, guinea pigs and dogs by means of aleurone grain injections. This was withdrawn and mixed with emulsions of tubercle bacilli; the mixture was then incubated. Films were made and also inoculations. Bartel and Neumann

conclude that the bacilli are taken up by the polymorphs in the first instance. After 24 hours to three days the polymorphs are gradually replaced by mononuclear forms until in from three or five days they completely disappear. The polymorphs again appear when caseation has occurred. With regard to the effect of the leucocytes upon the virulence of the organisms, no weakening of the tubercle bacilli was observed and there was no difference in virulence between bacilli treated with exudate where polymorphs predominated and those treated with the mononuclear exudate. They conclude that the taking up of the bacilli by the cells is of importance only in so far as these cells are the means by which the organisms are carried to the lymph glands, where the true struggle between the tubercle bacillus and its poisons is carried out.

The experiments which I have conducted for the present enquiry may be divided into (1) those carried out by inoculating animals with virulent turbercle bacilli and observing the cellular reaction, and (2) similar observations upon animals treated with doses of tuberculin.

(1.) *Inoculation Experiments on untreated animals.*

A series of six rabbits was inoculated with $2\frac{1}{2}$ cc. of an emulsion of a strain of tubercle, obtained from the mesenteric glands of a child, which showed the characters of the "typus humanus." At intervals of 24, 48, 72 hours, 6 days and 10 days the animals were killed; fluid was drawn off from the abdomen and examined on the warm stage and in films stained by various methods. The omentum was removed, fixed, spread out and examined. Portions were also cut in paraffin. Two other rabbits were also inoculated with a strain with the characters of "typus bovinus"; they were killed at intervals of 8 and 24 days. The methods for staining used were, for films, Leishman, Jenner, and combinations of these with bacillary stain; the bacillary stain used was carbol- or anilin-fuchsin (two minutes steaming; decolourisation with 1 per cent. HCl in methylated spirit). Sections were stained for half-an-hour in carbol- or anilin-fuchsin in paraffin bath and counterstained in Leishman or in methylene blue.

The following is a summary of the findings:—

24 *hours after inoculation.*—Types of cells present are as follows:—

1. Polymorphonuclear amphophils.
2. Small mononuclear cells corresponding in appearance to the lymphocytes of the blood.
3. Larger mononuclear cells with larger nucleus and considerable amount of protoplasm which is often vacuolated.
4. Cells with the characters of endothelial cells but rounded and with vacuolated protoplasm. No hard and fast line can be drawn between (3) and (4).

The predominant type present in the exudate is the monuclear of larger type although polymorphs are abundant, especially in the omental tissue. Tubercle bacilli are relatively few in number and may be seen in the polymorphs but in larger numbers in the free mononuclears and fixed endothelial cells. Marked phagocytosis of larger mononuclears for polymorphs.

48 *hours after inoculation.*—Same types of cells observed. Larger cells observed on warm stage have markedly branched and vacuolated protoplasm, the processes uniting with others from similar cells. Spread omentum already shows aggregation of cells into groups, some of which contain masses of bacilli. Where bacilli occur in masses they are always surrounded by polymorphs, but when isolated they occur chiefly in the larger mononuclears.

72 *hours after inoculation.*—A more marked reaction is to be observed. Large numbers of medium sized mononuclear cells with vacuolated protoplasm frequently containing bacilli. Some of these are bi-nucleated, and occasionally the nucleus may show mitosis. Groups of these cells are to be observed on the surface of the omentum.

Marked infiltration of the omentum with cells, both polymorphs and mononuclears, more especially the latter, aggregated into groups. Surface cells show evidence of multiplication, many of them being bi-nucleated.

Bacilli found in the fixed endothelial cells, also in rounded mononuclear cells.

6 *days after inoculation.*—Lymphocyte-like cells are now more numerous, polymorphs relatively few in fluid, more numerous in omentum. Distinct aggregation of cells into clumps, many of which have giant cells in their centres. Bacilli are fairly numerous and occur chiefly in the giant cells. Cells around are mainly mononuclear forms resembling large lymphocytes. A considerable number of polymorphs may be seen scattered through them. Mitotic figures are not numerous but may be seen in the fixed endothelial cells. Large mononuclears free on surface found containing bacilli.

10 *days after inoculation.*—Cells of lymphocyte type both large and small predominate, but polymorphs again numerous. Some of the larger mononuclear cells are bi-nucleated and show all stages of division by direct method. Omentum greatly thickened by infiltration with cells. These are aggregated into foci in the centres of which areas of commencing caseation can be seen. In these centres polymorphs are specially numerous. Cells making up aggregations are large branched mononuclears (epithelioid cells); bacilli are very numerous in them. Some cells multinucleated forming fairly typical giant cells, also containing bacilli. In caseous areas bacilli occur in polymorphs and also free. Surface endothelial cells show evidence of active multiplication. Small lymphocytes present in tissue but not very numerous.

In another animal inoculated at the same time and killed after 10 days the appearances were precisely similar.

In the animals inoculated with the bovine strain the appearances resembled those found in animals killed 10 days after injection of the human strain. In the case of animal killed after 24 days caseation was more advanced.

Another serious of experiments was performed by inoculating three guinea pigs with bovine tubercle bacilli. The peritoneal exudate was withdrawn and examined at intervals of 5, 24, 48 hours, 3 and 7 days. The animals were killed after 7, 13, and 20 days; the fluid was withdrawn and examined, the omentum was spread out,

stained and examined, and sections of omentum, abdominal glands, and other organs were made and stained. As regards staining methods used, in addition to those previously mentioned Much's modification of Gram's method was employed in every instance to compare with the carbol-fuchsin-Leishman preparations.

Two and a half cc. of the emulsion were in each case injected. Stained by Much's method, the emulsion showed apparently all the bacilli black but with distinct gaps, some being swollen and club-shaped. The counterstain used was dilute fuchsin, but there was no evidence of any bacilli being stained with it. Specimens stained with carbol fuchsin showed the bacilli much more homogeneous, and where granules were seen the intervening portions of the organism still stained faintly pink.

After five hours the predominant cell is undoubtedly the polymorph. Many of these contain bacilli, some are filled with them. Mononuclear forms are relatively few, a few of these have the appearance of free endothelial cells. The majority are fairly large rounded cells with a large amount of finely granular and vacuolated protoplasm. Many of these contain bacilli. None of the mononuclear forms show a tendency to take up polymorphs. There are very few free bacilli. Stained with Much's method the bacilli within the cells appear to be slightly more granular. The number of coarsely granular eosinophils varied in different animals.

After 24 hours the mononuclear cells are distinctly more numerous. A large number of these appear like free endothelial cells. They are large cells with abundant vacuolated protoplasm staining faintly pink. Polymorphs are still abundant and contain many bacilli. Many occur within mononuclear forms. All the mononuclear forms show marked phagocytic properties as regards the bacilli. Cells resembling small lymphocytes are fairly numerous and are occasionally to be seen inside large mononuclear forms. Much-stained specimens show no change in the appearance of the bacilli.

After 48 hours there is a further increase in the number of mononuclear cells. Some are obviously free endothelial cells. These show marked phagocytic activity for polymorphs and for bacilli. Other cells are smaller and rounded with protoplasm which takes on a bluish tinge. These also show marked phagocytic activity. Small lymphocytes are now fairly abundant, but no very sharp line can be drawn between these and the previous variety. Polymorphs are still fairly abundant but few now contain bacilli. No bacilli with characteristic staining reaction are found free, but bodies resembling organisms and staining faintly blue or pink are found free. Probably these are the bodies regarded by Markl as degenerating tubercle bacilli, but in the absence of distinct gradations between these bodies and bacilli staining in the usual way it would be rash to draw such a conclusion. In specimens stained by Much's method there is some evidence of increased granularity in the bacilli within the cells.

After three days the larger mononuclear cells predominate. There are a few distinct endothelial cells, but the majority are of the round vacuolated type, the protoplasm of which takes on the basic stain. Bacilli are fairly numerous in these, and with Much's method they appear to be somewhat more granular. Phagocytic activity of the large mononuclears for other types of cells is not so marked. Small lymphocytes occur in fair abundance. Some, although not the very smallest, contain bacilli.

After seven days the most frequent cell is now the smaller mononuclear, cells resembling lymphocytes or a little larger. Cells resembling endothelial cells are not frequent. Polymorphs are few in number. Bacilli are few in number, and occur only in the larger mononuclear cells. Stained by Much's method, the bacilli appear very granular.

After 13 days the cells are practically all of the mononuclear type, The vast majority consist of cells with relatively large round dark staining nuclei and a very small ring of finely granular protoplasm. These vary, however, in size and forms intermediate between these and the large endothelial-like cell can be seen.

After 15 days the appearances are very similar, but a few more polymorphs can be found. There are also more of the large vacuolated type of cell, and these show phagocytic activity for small lymphocytes. Tubercle bacilli are present in small numbers in the larger cells.

Summary of Experimental Results.

The presence of the tubercle bacillus in the animal body incites to the production of an exudate, the cellular elements of which are, in the first instance, chiefly polymorphonuclear leucocytes. These rapidly take up and ingest the bacilli. Mononuclear cells are, however, also present; many of these are leucocytes, others are shed endothelial cells. Both these varieties also act as phagocytes. After 24 hours the chief cellular element becomes the mononuclear cell. Some of these are undoubtedly free endothelial cells; they are branched and vacuolated. There is also evidence of the multiplication of these cells. They are actively phagocytic and take up bacilli and also polymorphs. Fixed endothelial cells also act as phagocytes. From 24 hours onwards the polymorphs become steadily less numerous in the exudate. They are, however, still in evidence in the tissues. After three days the smaller mononuclear cells resembling lymphocytes are present in large numbers, and these increase in numbers until after seven days, when they form the predominant type of cell. These cells do not act as phagocytes, but cells with a similar appearance, though with more protoplasm, are undoubtedly phagocytic. It is probable that the lymphocyte is capable of growing into the larger form. The endothelial type of cell persists, although in much reduced numbers. The bacilli gradually disappear both in the cells of the exudate and in the tissue. There is no evidence, however, that they are destroyed within the cells. They certainly become more granular within them, but do not lose their staining reaction entirely. Even within the peritoneal cavity there is a tendency for the cells to aggregate and form follicles.

Subsequent to the primary reaction there follows a period during which bacilli are few and far between, and the cellular reaction in like manner subsides. During this period the bacilli which are found occur in the middle of aggregations of cells of mononuclear type, sometimes within giant cells.

During and after this quiescent period, the duration of which depends upon the virulence of the organism and therefore varies with the strain of the germ employed, the cells present are chiefly of the small lymphocyte variety, but when caseation commences in the subjacent tissue polymorphs are again in evidence.

In the tissues of the omentum subjacent to the endothelium, mononuclear cells of similar appearance to the endothelial cells eventually take up the bacilli. They enlarge, become branched, and tend to aggregate together into follicles. Either by multiplication of nuclei or by fusion of cells in certain cases, depending largely upon the strain of the organism, giant cells are formed. Later on, when caseation and breaking down of tissue takes place, again marked increase in the polymorphs occurs. They again show active phagocytosis for bacilli and suffer inclusion from larger phagocytes. There is no evidence that by Much's method forms of the organism are demonstrated which cannot be shown by the older methods.

It has been stated by some authors that the primary leucocytic reaction is merely the reaction to the bacillus as a foreign body, and that there is nothing specific about it. That this is not the case is shown by the variation to be observed in the way in which phagocytes ingest the organisms in opsonic estimations. The tubercle bacillus carries with it specific toxins which are operative at once in modifying the reaction following their introduction into the tissues.

In the foregoing observations no attempt has been made to distinguish between the endothelial cells lining the peritoneal cavity and endothelial cells and connective tissue cells of the omentum itself. This is because I believe with Schott (1909) that no such distinction can be made. To use this author's language, "in inflammatory processes of the peritoneum all the sessile cell elements of the peritoneum are concerned. No distinction can be made between the covering cells and the subjacent connective tissue cells."

(2) *Experiments on Animals treated with Tuberculin.*

Among the first to publish experimental results with tuberculin were Arloing, Rodet, and Courmont (1892). According to their observations no arrest of the tuberculous process occurred in inoculated animals. On the contrary the treatment appeared to favour the multiplication and extension of the tuberculous process. As regards the histological results of treatment, they observed congestion, inflammatory exudation, chiefly of lymphatic cells in the alveolar walls but also of fibrin and of catarrhal cells into the alveoli themselves ; in addition, they found hyaline degeneration of the epithelial cells and formation of new tubercles (pp. 47 and 74).

Klebs (1894) in an exhaustive study of the effect of tuberculin on animals states his histological findings as follows :—The resolution of tuberculous lesions which follows this treatment with tuberculin is accompanied by a slight inflammatory reaction consisting in dilatation of vessels, exudation of fluid and emmigration of a few leucocytes. This very slight leucocytosis which accompanies a properly conducted course of tuberculin treatment provides the necessary material for the restitution of the tubercle cells, notably nuclein (p. 247). The too liberal use of raw tuberculin provides an inflammatory reaction which is damaging and leads to the dissemination of the disease (p. 219).

Broden (1899) inoculated tuberculin (Denys), sometimes before sometimes after inoculation of emulsions of bacilli, into the peritoneal cavity. He concludes that the tubercle neoplasm is much

more developed in the treated animals; in other words the defence is much more energetic. In the treated animals the bacilli remain few in number, isolated in the cells. Sometimes they stain badly, become granular and disappear. There is no essential difference between the reaction in animals treated with tuberculin. The tuberculin merely reinforces the defensive powers of the body.

The following are the results of my own experiments :—

First Series.—Three animals were inoculated with ·1 cc. of tuberculin, a dose which Klebs states produces a definite degree of protection, and after 24 hours these and three untreated animals had each 2½ cc. of a suspension of bovine bacilli injected into the peritoneal cavity. Fluid was withdrawn at intervals of 5, 24, 48 hours, 3 days, and 7 days, and the animals killed after 7, 13, and 20 days respectively, and the tissues examined carefully. In every case in addition to ordinary staining methods Much's modification of Gram was used.

It may be stated at once that in the early stages no distinct difference was to be observed between the two sets of animals. In the later stages the differences were not sufficiently marked to call for notice.

Second Series.—A guinea-pig which had been injected with bovine tubercle bacilli eight days previously was given a dose of ·1 cc. of old tuberculin (Lucius and Brüning, Höchst a/m). The characters of the peritoneal exudate were examined before injection and at intervals of 4, 8, 24, 32 hours and 48 hours after, and at the same time compared with an animal inoculated with bacilli but untreated with tuberculin. In every case Much's method was used in addition to carbol-fuchsin. At the same time the temperature of the animal was taken.

Before inoculation the cells were chiefly of the small lymphocyte type. Endothelial cells and large lymphocytes were also present, but no polymorphs. No bacilli were found.

Four hours after there is a distinct increase in the number of polymorphs. The large mononuclears show evidence of phagocytic activity towards the leucocytes.

Eight hours after the number of polymorphs is still more marked.

Twenty-four hours after.—Small lymphocytes are still numerous. There is a distinct increase in the large mononuclear type of cell. These are markedly vacuolated. One of these was found in process of mitotic division. Polymorphs are present in abundance and eosinophils are also numerous. No bacilli were found.

Thirty-two hours after.—The predominant cell is now the large vacuolated (endothelial) cell. Many of these have two nuclei. A few tubercle bacilli were found in these cells after careful search. Polymorphs are relatively not so numerous; eosinophils are also few in number.

Forty-eight hours after.—Lymphocytes, both small and large, are the chief type of cell present. There are a fair number of polymorphs, but they are not nearly so numerous. No bacilli have been found.

A second guinea-pig was inoculated with 1 cc. of bovine tuberculin, 17 days after inoculation with an emulsion of bovine bacilli. Samples of peritoneal exudate were removed by needle as before. Again a pronounced activity on the part of the polymorphs and, later, on the part of the endothelial cells was observed.

A rabbit, inoculated with bovine tubercle emulsion 17 days previously, had ·03 cc. old tuberculin injected into the right groin. Samples of peritoneal exudate were withdrawn at the same intervals as before, with very similar results to the above. A similar increase in the number of polymorphs in the exudate was observed.

A third series of experiments was carried out with a view to determining whether any difference was observable in the inflammatory reaction to tubercle bacilli in animals which had been immunised by a course of tuberculin treatment and animals which had not been so immunised.

The method of immunisation was that adopted by Klebs, apparently with success. Three guinea-pigs were inoculated with ·04 cc. tuberculin R. The following day a similar dose was given. Two days later a dose of ·1 cc. was given. Four days after, a dose of ·2 cc. was given to two of the animals. One was observed to be disinclined to move. It was killed with chloroform and examined carefully. Nothing of note was found.

Four days after the last injection the two remaining guinea-pigs, weighing respectively 365 and 390 grammes, were inoculated with 2½ cc. of an emulsion of tubercle bacilli obtained from a case of mesenteric and pulmonary tuberculosis in the human subject. At the same time two other guinea-pigs, weighing respectively 280 and 350 grammes, were inoculated with the same dose.

Samples of the fluid from the peritoneal cavities were removed at intervals of 24, 48 hours, 4 days and 6 days.

After 24 hours the chief cells present were the polymorphs, many of which contained bacilli. Large mononuclear cells were also present showing vacuolation and phagocytic activity towards bacilli. No difference was observed in the immunised animals.

After four days the mononuclears were the predominate cell, and bacilli were found chiefly in them. These cells also showed marked phagocytic activity for the polymorphs. In the unimmunised animals the polymorphs appeared to be more numerous. The bacilli were found to be still present in them in abundance. There was also some difference as regards the number of bacilli present, the untreated animals showing much greater numbers. Little stress can, however, be laid upon these variations owing to the considerable difference in the weight of the animals.

Summary of results obtained in animals treated with tuberculin.

No difference is to be seen in the cellular reaction to the tubercle bacillus in animals treated with a single large dose of tuberculin, nor in those treated previous to inoculation with a series of doses increasing in strength. If, however, an animal already infected be treated with tuberculin, a marked increase in the polymorphonuclear reaction is to be observed with a corresponding increased phagocytic activity of mononuclear forms for polymorphs.

There is also increased activity and probably a multiplication of the fixed tissue cells concerned in the formation of the tubercle granulations. Some of these cells containing bacilli may, in the process, be set free.

General Conclusions.

1. The tubercle bacillus is in the first place largely taken up by the polymorphonuclear leucocytes. Subsequently the mononuclear cells, which are to a considerable extent of endothelial and connective tissue origin, take up the polymorphs and also ingest bacilli on their own account, hence the bacilli come to lie within such mononuclear cells. These develop into the so-called endothelial cells of the tubercle follicle. This is true of the later stages of the disease as well as of the earlier. The more acute the disease the more marked is this phagocytic activity of the polymorphs. When masses of bacilli occur in a tissue, the cells in immediate contact with them, if there be any such, are polymorphs.

In other words the cellular reaction is the same in tuberculosis as in other infective conditions, the polymorphs forming the first line of defence. The connective tissue cells are prominently present as a rule, because of the relatively slow action of the poison.

As regards the large mononuclear cells of the blood, these also show phagocytic activity, but in the later stages of the reaction it is impossible to distinguish these from cells of the connective tissue type.

2. There is no obvious variation from this sequence of events to be observed in animals treated with single doses of tuberculin or in those which have been "immunised" by repeated doses of tuberculin. On the other hand the administration of a single dose of tuberculin in an animal already infected with the disease has the result of considerably increasing the polymorphonuclear and probably also the connective tissue reaction in the infected area.

3. Bacilli ingested by phagocytes appear more granular, especially when stained with Much's method. There is, however, no evidence in active tuberculosis that a form of the organism is stained by this method which cannot be demonstrated by the older methods. Further, there does not appear to be ground for believing that within the phagocyte the bacillus undergoes a change whereby it loses entirely its power to fix aniline dyes.

4. In animals treated with tuberculin there is no obvious increase of the number of granular bacilli as demonstrated by Much's method or by the older methods when compared with the number seen in untreated animals.

The author wishes to express his gratitude to Professor Leith of Birmingham University and to Dr. James Ritchie of the Laboratories of the Royal College of Physicians, Edinburgh, for their kindness in allowing the foregoing work to be carried out in their laboratories.

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A Contribution to the Study of the Sub-Chorial Hæmatoma of the Decidua (the Blood Mole).

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A Contribution to the Study of the Sub-Chorial Hæmatoma of the Decidua (the Blood Mole).

By Alice M. Hutchison, M.D. (Edin.).

(Abstract of a thesis for the degree of M.D. of Edinburgh University in 1905.)

During a year's work at the Hospice, 219, High Street, Edinburgh, my interest in the subject of blood moles was aroused by the fact that five such specimens came into my hands. Two have since been sent to me for examination, making a total of seven. I was still further interested, and stimulated to make a careful study of these specimens with a view, if possible, to throwing some fresh light on the subject, by finding that four out of the seven cases had to be curetted on account of continuance of symptoms. A microscopical examination of the curettings was unfortunately only made in two of them.

Before entering upon a description of these cases, it is intended to review very shortly the various theories that have been put forward up to the present time as to the causation of blood moles; these having been gleaned from the literature on the subject. Most of them are based upon no proper investigation, and are of little value.

The earliest writers on the subject date from the time of Hippocrates and Galen, and amongst later ones must be mentioned Mitschik, Müller, Scanzoni and Perenice. Of still more recent writers the most noteworthy are Jakobson, Schwalbe, Spiegelberg, Leditsch and Galabin (1891). Breus, however, in his paper published in 1894, was the first to give a detailed description of these sacs and a definite theory, formulated as the result of his investigations. The variety characterized by the presence of many polypoid blood effusions he believed to be quite distinct from ordinary blood or fleshy moles and to originate in the following way:—First, death of the fœtus occurs from unknown causes about the second month. The fœtal membranes, however, continue their growth, and, finally, owing to the lack of any expanding pressure, fold inwards and form diverticula which are rapidly filled by blood effusions, the late involvement of the decidua and consequent long retention of the ovum being thus explained. This theory Breus formulated as the result of a careful naked-eye investi-

gation of the diverticular membranes. Three points about them he emphasized especially, as proving that they are preformed sacs and not mere projections of the amnio-chorion through blood effusions: —

1. Their extremely complicated shapes and stalks.

2. The presence of empty sacs, which show no sign of having had blood in them.

3. The fact that the membranes, when emptied of blood-clot, retain their various shapes and show strands of connective tissue.

Neumann, in 1897, attacked Breus's paper, and a lively discussion followed, Breus's mole being held by many to be in no way different from an ordinary fleshy mole, and the possibility of further growth of the membranes doubtful. Neumann threw a little light on the subject by suggesting some anomaly, maternal or fœtal, as the cause of the hæmorrhages, and through them of the death of the fœtus, without, however, discussing the possibilities in this line or their mode of action.

Gottschalk now stepped in as a mediator, but since his view is practically Breus's, with the added suggestion of insufficiency of the primitive heart germ as the cause of fœtal death, it is unnecessary to give it in detail.

In 1891 Davidsohn's paper appeared. In this the author expressed his belief that the striking misproportion between fœtus and sac wall is not apparent but real, and since this does not originate after the death of the fœtus (Breus's theory of further growth of the membranes being quite untenable), the conclusion must be that it was already present at the time of death. The only feasible solution is, therefore, that hydramnios of the ovum is the cause of blood moles. Davidsohn considers that only by some such theory as this is it possible to explain how an ovum of a circumference of only a few centimetres can reach a circumference of 10 centimetres through the formation of subchorial hæmorrhages, without uterine contractions being set up. Also, to regard the disproportion between fœtus and sac as simply a result of passive pressure through hæmorrhages, he considers to be entirely disproved by the fact that the chorionic membrane is thickly set with villi, and if by chance these are thinned out in one part they are proportionately thicker on neighbouring parts.

Taussig and Engel may be mentioned as exponents of the theory of a secondary hydramnios. As a possible cause of it they mention endometritis or some other form of genital disease, since this was present in some of their cases.

Bauereisen's view, though quite untenable, may be shortly mentioned. He believes that deported villi in or pressing on

decidual veins, block the circulation and cause a stasis. The fœtus dies from lack of nourishment and hæmatoidal swellings develop owing to accumulation of blood in the sinuses. Bauereisen formulated this theory upon the fact that in his microscopical examinations he found what he took to be deported villi in the deep decidua, *i.e.*, villi having no connection with the amnio-chorion.

The first British writer on the subject is Dr. Berry Hart, and his theory of origin is as follows:—There is a blocking of the serotinal sinuses in the large-celled layer, which is an exaggeration of the physiological thrombosis of these sinuses observed at different stages of pregnancy by different observers, Dr. Gulland and himself in this country and Friedländer and Leopold in Germany. A slow engorgement of the intervillous circulation results, and the amnio-chorion is bulged between two chorio-basal septa. In the microscopical examination of his specimen, Dr. Berry Hart found a curved chorio-basal septum persisting at the sulcus bounding the base of the tuberosities, with a blocking of the thin wall sinuses in the large-celled layer of the serotina. The embryo dies on account of interference with its circulation, and the thrombosed mass is expelled after a certain time.

Having now put forward the main theories offered, up to the present time, as to the causation of blood moles, it will be most convenient to give here the details of the seven cases mentioned. The defects in previous theories, and the necessity for a fresh view of the subject, will then be considered.

Clinical Histories.

Case I., æt. 27, i.-para. Patient had a normal confinement a year after her marriage, the child not being nursed. During the following two years there was first amenorrhœa for nine months, followed by periods of combined amenorrhœa and irregular hæmorrhages, ending in an abortion. After the abortion she was treated for persistent bleeding. Six months later she sought advice for floodings following on a three months' period of amenorrhœa, the uterus being enlarged to about the size of a two months' pregnancy, and the fornices clear. After an interval of two months the blood mole to be described was passed. Patient turned up again nine months later, complaining of irregular hæmorrhages since the miscarriage, and she was curetted. Regular periods then followed for eight months, during which time she was in attendance at the Victoria Dispensary. Three months later she had an abortion, developed pneumonia, and died.

Case II., æt. 31, iv.-para. Patient had two miscarriages between

the first and second child. After the death of the fourth child at the time of weaning, amenorrhœa continued for three months, when the sac to be described was passed. For three weeks afterwards she bled at intervals, on exertion or with any excitement. Two normal periods followed, and three months later a second blood mole was passed.

Case iii., ix.-para. Patient had six normal pregnancies in succession, followed by a miscarriage. Again three normal pregnancies and a second miscarriage, followed by profuse periods and an inter-menstrual discharge which became coloured with any unusual exertion or excitement. A third miscarriage (blood mole) occurred after two months' amenorrhœa, and, in spite of the fact that the sac came away entire, patient was curetted on the third day on account of hæmorrhage.

Case iv., æt. 39, viii.-para. Patient had eight normal confinements, then a miscarriage. A second miscarriage (the blood mole to be described) occurred four months later.

Case v., æt. 32, vi.-para. Patient had first two normal confinements, then a miscarriage, after which she was curetted on account of bleeding. Again two normal confinements, then a premature birth at the sixth month, when the placenta was adherent, and had to be manually detached. Two normal periods, then after nine months' amenorrhœa the blood mole to be described was passed.

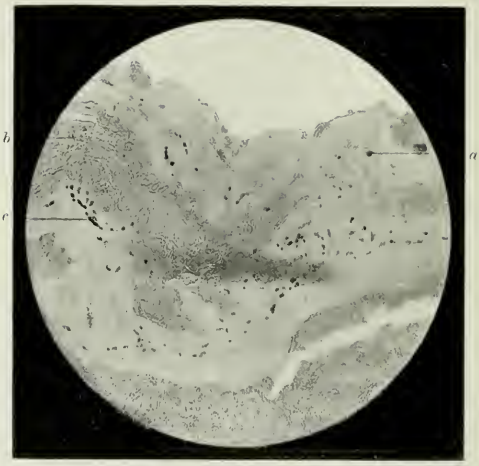
Case vi., æt. 28, v.-para. Patient had five normal confinements, and after weaning the fifth child a flooding which lasted a week. Two months later the blood mole miscarriage occurred. About a month afterwards she reported herself as having irregular bleeding, and was therefore curetted. A normal pregnancy and confinement followed.

Case vii., æt. 32, ii.-para. Patient had first two normal confinements, then after nine months' amenorrhœa, the blood mole to be described was passed. Patient stated that when $4\frac{1}{2}$ months pregnant she got a fright from a dog, and a dark-coloured discharge began, which lasted for two days, then stopped for a time and returned again for one day.

Description of Sacs.

To simplify this description the points of striking similarity between the sacs will be described, with, at the same time, a mention of the differences noted.

1. They were all (with the exception of No. vii., which had a definite placenta) closed sacs of greatly thickened decidua.



Ed. Muir.

Fig. 1.

(*a*) Pigmented cells and granules stained with ferrocyanide of potassium and lying in hyaline degenerated amnio-chorion and villi.

(*b*) Fibrin.

2. They varied in size, from a 2—3½ months' pregnancy, and the placenta of No. vii. was that of a 5—5½ months' pregnancy, with a three months' fœtus.

3. Sacs Nos. i., iii., iv., v., and vii. contained a very small amount of liquor amnii, and Nos. ii. and vi. what seemed an unusually large amount, and of a glistening appearance.

4. Hæmatomata were present in all except Nos. iii. and iv., and varied in appearance from large soft sacs to solid finger-tip projections or convolutions.

5. A cross section of the wall in all the cases had the appearance of blood clot in various stages of organisation, judging from its consistence.

6. There was no appearance of a fœtus in any sac except No. vii., though some fragments escaped with the liquor amnii in No. ii.

7. Soft brown material, like altered blood clot, was lying on the amnion in Nos. i., v., and vii.

Microscopical Characters.

On account of the great similarity between the specimens it will be sufficient to describe one case minutely, and indicate points of difference in the others.

Case i., Fig. 1. *Low power.* The epithelium of the amnio-chorion shows as a broken granular-looking line which is stripped off in parts. The connective tissue appears hyaline and thickened in parts, and pigmented cells and granules are present in quantity. Many projections and infoldings of the amnio-chorion are seen. The amnio-chorion rests on fibrinous clumps and strips, with a few hyaline degenerated villi embedded in them, pigment granules being present here also (stained with ferro-cyanide of potassium). Below this is fairly recent-looking hæmorrhage, shading for the most part gradually into deeply-stained fibrin with hyaline degenerated villi lying in it. There is no appearance of anything that can be described as decidua, and there are no vessels.

High power. Amnio-chorion. The epithelium of the amnion, and of the chorion where it can be distinguished, is extremely granular; the connective tissue varies in appearance from wavy, fibrous tissue to stretches of hyaline degeneration; many cells are in it, pigmented or non-pigmented, and of various shapes and sizes. The hæmorrhage shows gradations from commencing coagulation to deeply-stained fibrin, in which are large spindle cells and granular pigment.

Villi. All show hyaline degeneration, with a granular appearance of the epithelium; they have no vessels.

Other specimens showed a definite intervillous space and decidua. The decidua was either œdematous or torn up by hæmorrhage in various stages of organisation, with, in most cases, a leucocytic infiltration, and in some necrosis. The villi in all cases were degenerated (myxomatous or hyaline), and seldom showed any sign of vessels. The amount of syncytium on them was in all cases abnormally small. The intervillous space showed a thrombosis, either filling it or limited to one end, and pressing on and obliterating the villi embedded in it (Fig. 2). Vessels of various sizes were found in the decidua, but for the most part with a broken and altered outline, and some containing thrombi. Pigmented cells were found in all cases, either in the amnio-chorion or decidua, some staining blue with ferro-cyanide of potassium.

Description of endometrial scrapings from Cases i. and vi.

Case i., Fig. 3. *Low power.* The superficial epithelium has a broken outline. The glands are dilated, and some contain blood. The stroma has very extensive hæmorrhages into it; indeed, they have the appearance of replacing it over large areas, with stroma cells, glands and blood-vessels scattered through them. The number of the thin-walled vessels is greatly increased, many of them showing considerable dilatation.

High power. These points are confirmed.

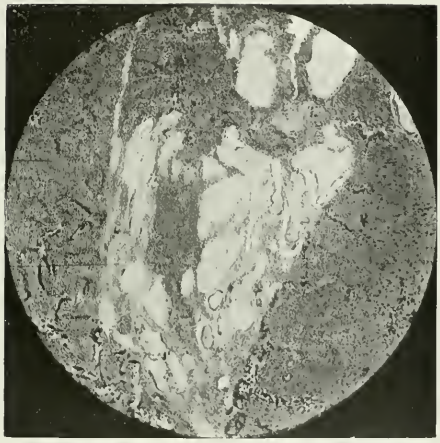
Case vi. *Low power.* In parts there is a very marked increase of the interstitial tissue, which appears in places to be pressing upon and destroying the glands. In other parts the increase is less marked, or the stroma appears to be even rarefied. There is a multiplication of the thin-walled capillaries, many of which are greatly dilated. Extensive hæmorrhages are scattered through the stroma in every part of the slide.

High power. Confirms these details, and shows very plainly the destruction of the glands by the increase of interstitial tissue.

Since any theory to be practicable must be capable of explaining not one variety only but all varieties, the conditions universally present may be thus summarized: —

History. Amenorrhœa, followed by the expulsion of a blood mole at any time up to or even beyond the limits of a pregnancy, with or without intervening hæmorrhages.

Sac. Its size may be strikingly out of proportion to the period of pregnancy, or may fairly well correspond to it. The wall may



Rd. Muir

Fig. 2.

(*a*) and (*b*) Degenerated and compressed villi.
(*c*) Thrombosis in intervillous space.

Rd. Muir.

Fig. 3.

(*a*) Hæmorrhage in interstitial tissue.
(*b*) Dilated thin-walled vessel.
(*c*) Dilated gland.
(*d*) Nuclei of stroma cells.

only be greatly thickened or show hæmatomata of any of the varieties mentioned. A cross section of the wall has in all cases the appearance of blood-clot, recent or organising.

Fœtus. It may be entirely absent, or if present is well preserved, at a lower grade of development than the sac, and out of proportion to the date of pregnancy.

Microscopical appearances. Thrombosis in the intervillous space, with degenerated villi, decidua œdematous or destroyed by hæmorrhage, pigmented cells, and other details as described.

How have previous theories failed to explain all these conditions?

Breus's theory, even allowing the possibility of further growth of the membranes, is only applicable to a small number of blood moles (those showing hæmatomata of all shapes and sizes), and is therefore untenable, since a microscopical investigation proves all forms to be structurally alike.

Davidsohn's theory, like Breus's, can only apply to the very marked forms of blood moles, and for this reason alone it is unsatisfactory, indeed untenable. It seems also difficult to imagine that in each case the hydramnios is present for a time only, and then disappears without any renewal, and yet with the pathological conditions originally causing it still present.

Bauereisen's view is so entirely improbable that there is no need to say much about it. It need only be pointed out that deportation of villi, according to Poten and others, occurs in every pregnancy, a lodgement in some cases being first found in the minute lung capillaries. The impossibility of demonstrating deported villi in decidual veins without reducing the whole thickness of the wall to serial sections, may also be mentioned.

Dr. Berry Hart's theory strikes one as rather unsatisfactory, since it stops short of suggesting any cause for the undue blocking of the serotinal sinuses. What are the altered conditions which have caused a physiological process to become pathological?

While quite recognizing that in the theories reviewed there is more or less of truth, it yet seems, from a study of these seven cases, that there is need for a fresh view of the subject, which especially directs attention to the endometrium as the most important factor in the production both of a normal fœtus and an abortion sac. Taussig and Engel, as has been already indicated, have vaguely suggested this, without attempting to work it out. Dr. Berry Hart has touched on it by his particular study of the condition of the

sinuses in fleshy moles, and three authors—Gottschalk, Taussig and Bauereisen — have described endometrial scrapings after blood mole, without, however, offering any special comment. On comparing the histories of the present cases the prominent feature was undoubtedly the existence of an unhealthy endometrium in conjunction with the production of these moles. The recognition of this fact led naturally to a study of the normal endometrium in relation to the implantation and development of the human ovum, and of abnormal endometria, more especially such as might be supposed to have a bearing on the production of blood mole. Space allows of only a short mention of a few of the most important points gathered from this study, more especially those which must be emphasized in any attempt to connect blood moles with an unhealthy endometrium.

1. The very important part played by the capillaries of the endometrium, which dilate during the first three months of pregnancy to form large sinuses, communicating, on the one hand, with the intervillous space, and on the other, with the tortuous arterial vessels.

2. The gradually effected communication between the sinuses and the intervillous space by the extension of processes of trophoblast and syncytium into them.

3. The very important part played by the syncytium in the nourishment of the fœtus and the necessity, in order efficiently to perform this part, that prolongation of syncytium should draw upon fresher maternal blood from the deep decidua, and that the blood stream through the intervillous space should be reduced to a slowly moving mass.

Nature appears by these means to have done everything in her power to reduce to a minimum the shock of any sudden change in the maternal circulatory system, in order that the villi may retain their hold upon the decidua and time be given for the interchange of food stuffs and gases between fœtal and maternal blood.

Pathological variations of the endometrium are very great and very numerous, and so it is only possible to touch very briefly upon those which might be supposed to have a more direct bearing on the formation of blood moles, that is, those showing a change in the vascular conditions, since hæmorrhage is the characteristic feature of all blood moles.

1. Glandular endometritis, which is by far the commonest variety met with. It is characterized by œdema of the stroma, enlargement

of the glands, and increase in the number and size of the thin-walled capillaries, with a ready tendency to hæmorrhage.

2. Sarcoma-like changes in the interstitial tissue which indicate either an early sarcoma or a simple hyperplasia. There is in this variety also an increase of the thin-walled capillaries, with a ready tendency to hæmorrhage.

3. Arterio-sclerosis of the vessels of the endometrium and of the entire uterine arterial system, which is, however, not a common form. Its characteristic feature is severe and intractable hæmorrhage.

Before entering upon the details of a fresh theory on the lines indicated, it will be well to attempt an answer to the most obvious objections.

1. Why should an abnormal endometrium cause a blood mole in one woman and an ordinary abortion sac in another, or the two in succession in the same woman?

2. Would one not expect every case of blood mole abortion to be associated with definite symptoms of endometritis?

Each writer in turn appears to have started with the assumption that a blood mole is entirely different from an ordinary abortion sac, whereas the study of these seven cases has served rather to bring out the points of resemblance, and to impress most forcibly the necessity for microscopical examination of all abortion sacs as the only way in which one can hope to arrive at any understanding of blood moles.

The suggestion that blood moles are simply a form of abortion sac seems to find considerable support from the fact that hæmorrhage is the chief characteristic of both, the difference apparently being rather one of intensity, combined with a more gradual development and a later involvement of the deep decidua. In replying further to the second objection, it may be pointed out that there are many facts regarding menstruation and abortion for which no satisfactory explanation can be given. For instance, why does one woman abort on the smallest provocation, while another woman undergoes the most violent treatment and yet the pregnancy suffers no interruption? Or again, why should change of climate, work or circumstances cause either a temporary cessation of the menses or one or two very profuse periods? Further, it is a fact, not only as regards the seven cases mentioned, but also as regards the numbers reported by previous writers, that the overwhelmingly large majority are of the working class and multiparæ, a combination which does not lead one to expect a very normal endometrium.

Again, the question of curetting is always considered in a case

of repeated abortion or of sterility, where no adequate cause can be found, in spite of the fact that definite symptoms of endometritis may be absent, and when carried out it often leads to a healthy pregnancy.

There are then certain facts in the study of the present cases which appear to have a close connection with the formation of blood moles, but in attempting to interpret these facts as regards the actual method of production of blood moles, one is of necessity soon reduced to drawing deductions from them. The facts are as follows:—

1. Clinical histories pointing to an abnormal condition of the endometrium, associated with the expulsion of a blood mole.

2. Curettings of two of these cases confirming the clinical histories.

3. In two a normal pregnancy followed the curetting, in the third normal periods without a pregnancy.

4. The microscopical appearance of all blood mole slides, which are characterized by thrombosis in the intervillous space, with œdema of, or extensive hæmorrhage into, the decidua, along with degenerated villi and pigmented cells. In attempting to link these facts theoretically, it will perhaps be best to consider how any of the abnormal endometria mentioned might fail to respond to the stimulus of the growing ovum.

None of these endometria can be considered as likely to produce a normal implantation, in glandular endometritis more especially on account of the œdematous stroma and hypertrophied glands, and in the other two on account of the ready tendency to hæmorrhagic extravasations into the stroma. The ovum therefore starts life handicapped by an inability to effect a firm hold on the decidua. The influence of an imperfect union between ovum and decidua is so much the greater since, according to the most recent teaching, the ovum may be regarded as from its earliest days dependent upon the decidua for its nourishment, this being the most feasible explanation of the early established trophoblastic connection between fœtal epiblast and maternal blood. The failure of such an intimate connection would naturally lead to impairment of nutrition of the villi and a resulting imperfect development of the ovum.

As regards the condition of the decidua and intervillous space already described, the natural inference is that these are caused by the pathological capillaries, even though it is impossible to follow the details of the process. Since the decidua in some slides is almost destroyed by hæmorrhage and, in the best preserved, very œdematous, some villi will naturally lose their attachment and cease to be of

any use in the nourishment of the rapidly-developing ovum, since the syncytium depends upon its deep attachments for an efficient performance of its work. Since, again, no normal intervillous space is seen, but thrombosis compressing and destroying villi, with evidence found in the pigmented cells of the process having been continuous, many more villi must be thrown out of working order, and still less nourishment be carried to the rapidly developing ovum. It would naturally follow that a young ovum would die and undergo partial or complete absorption, while a more mature fœtus might die or continue its development imperfectly, so that at the time of expulsion its size would be entirely out of proportion to the stage of pregnancy.

In order to investigate the points as to whether in these uniformly thickened sacs placental differentiation was in progress or not, before the onset of hæmorrhages, slides were prepared in Cases i., ii. and iii. from two different portions of the sac, these naturally being chosen at random. In ii. and iii. it was interesting to find that in spite of the absence of any appearance of a placenta externally, the second set of slides prepared showed a strikingly larger number of villi, giving a much nearer approach to the appearance of a developing intervillous space. In Case i. no difference was found, but since ii. and iii. had demonstrated the possibility of placental differentiation being in progress, the investigation was not further pursued. It follows therefore, that in those cases where the hæmorrhages occur during the progress of placental differentiation, they are not limited to the placental site, but involve the decidua vera and reflexa, as would be expected since large sinuses are still present here. If the placenta is well differentiated they are naturally limited to it, as is well demonstrated in Case vii., where the placenta is seen as a thickened mass with well-marked solid hæmatomata, while the united decidua vera and reflexa are present as bands and shreds. In the first case the final result may be either a blood mole (of the type described by Breus or a less marked form) or a fleshy mole, the latter being simply a more organised variety of the former. Each of these again is either empty or contains a fœtus of imperfect development.

In the second case there is a fleshy mole as a placenta, with a fœtus of a proportionately much lower grade of development, as in Case vii., where the placenta is of about 5—5½ months, and the fœtus not more than three months.

The amount and distribution of hæmorrhage and thrombosis in each case will determine the presence either of well-marked polypoid hæmatomata, finger-tip projections or simply a uniformly thickened wall. The time of expulsion of the mass will depend upon the

involvement of the deep decidua, since then only does it become a foreign body in the uterus.

Summary.

As a result of these investigations the following explanation of the etiology of blood moles is offered:—

1. A pathological condition of the endometrium pre-exists (especially of its vascular system).

2. As a result, implantation of the ovum is less securely effected than normally.

3. A process of hæmorrhage into the decidua with thrombosis in the intervillous space occurs, whereby villi are detached and compressed in greater and lesser numbers, and the system of fœtal nourishment interfered with.

4. The embryo, if young, dies, and is partially or completely absorbed; if more mature it continues its development, but imperfectly.

5. The combined hæmorrhage and thrombosis lead to one or other form of sac described, dependent upon their extent and time of occurrence.

The bearing of the corpus luteum on the development of the ovum (as it has been worked out by Fraenkel) has not been overlooked, but of necessity can in these cases only be considered a theoretical factor, since no positive information is obtainable.

Many points in the formation of blood moles still lack a satisfactory explanation, and invite further and more searching investigation. The theory here presented is put forward in the conviction that so far insufficient attention has been paid to the endometrium, which is the most important factor in maintaining the nourishment of the ovum.

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XXXII.—On the Effects on the Metabolism of Chloroform administered by Different Channels.

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Preliminary.

Death under the administration of chloroform as an anæsthetic is an accident which, although fortunately rare, is well known to all; but the occurrence of a late poisoning, sometimes manifesting itself several days after the administration of the drug, is less well recognised. This too is rare, but its occurrence indicates the very profound effect which such a drug may exercise upon living tissues.

A very large amount of work has been done upon the immediate influence of chloroform upon the nervous system and upon the circulatory and respiratory mechanisms, but few observations on its more lasting effects upon the general metabolism have been recorded.

A protoplasm poison so powerful as chloroform might be expected to modify profoundly the chemical changes in the body. Upon the cells of the liver, after removal from the body, I found in 1893 that chloroform has a very marked action, accelerating the disintegrative necrobiotic changes (*Phil. Trans. Roy. Soc.*, vol. 185, p. 248), and at the same time accelerating the conversion of glycogen to glucose. That, during life, it has a marked effect upon the metabolism of protein, has been shown by Salkowski (*Virchow's Arch.*, Bd. 115, p. 339) and by Strassmann (*Virchow's Arch.*, 1889), both of whom found that its administration caused an increased excretion of nitrogen in fasting animals. Kast and Mester (*Ztsch. f. klin. Med.*, Bd. xviii., p. 469, 1891) also got similar results. Vidal (*C.R. de la Soc. biol.*, 1896, p. 474) records a series of observations on the excretion of nitrogen in the human subject before and after operations of different degrees of severity, in which chloroform had been administered, and he finds that, irrespective of the character of the operation, there is a rise in the excretion of nitrogen, when compared with its ingestion. He also investigated the question on fasting rabbits, and found that in these animals the excretion of nitrogen, after the separation of the albumin, is markedly increased by the simple inhalation of the drug.

Thompson (*Brit. Med. Jour.*, vol. i., 1906, p. 608) describes the result of a study of the immediate effect of the administration of chloroform and of ether upon the urinary secretion. His experiments extended over two to

four hours, and he states that with chloroform there is first a reduction in the volume and in the concentration of the urine secreted, followed by a rise in the amount. He finds that exudation of leucocytes and hyaline casts is apt to occur, probably due to the partial vascular stasis in the kidney, and that albuminuria occasionally is developed. Sometimes an increase in the reducing substances in the urine was observed.

The effect of chloroform upon the gaseous exchanges in respiration does not seem to have been studied experimentally. During the anæsthesia, Oliver and Garrett (quoted in Richet's *Dict. de Phys.*, vol. iii., p. 624) found an increase of carbon dioxide expired, and a decrease of oxygen absorbed, apparently as a result of the respiratory deficiency.

Present Investigation.

In 1900, when studying, with Dr Eason, the effect of various drugs upon the elaboration of urea in the liver, I intended to investigate the effect of chloroform, anticipating that its action would be similar to that of alcohol and sulphonal (*Jour. of Phys.*, vol. xxvi., p. 166, 1901). Other work interfered with the prosecution of these investigations. My attention was again directed to the subject by the appearance of a paper by Stiles and Stuart M'Donald on "Delayed Chloroform Poisoning" in August 1904 (*Scot. Med. and Surg. Jour.*, 1904, p. 97). In this an excellent *résumé* of the previous work on the subject is given.

The object of the present series of experiments is to investigate the effect of chloroform on the protein metabolism, to ascertain whether chloroform in any way influences the elaboration of urea in the liver, and whether its action is the same when administered by the respiratory passages, by the mouth, and hypodermically.

I.—General Plan of Investigation.

In this investigation the effect of the drug upon the chemical changes in the living matter of the body is deduced from the changes in the excretion of the products of disintegration of proteins in the urine: any *quantitative* change in the protein metabolised being indicated by changes in the total amount of nitrogen excreted per diem, while *qualitative* changes are indicated by alterations in the distribution of nitrogen and of sulphur in the different nitrogen- and sulphur-containing constituents of the urine. The animal was kept upon a fixed diet, and the average normal amount of these different constituents was determined for some days. Then the drug was administered, and their amounts were again investigated for a longer or shorter period, till any disturbance had passed off and the normal condition was again restored.

Since an interference with the usual course of protein metabolism is generally indicated by a change in the relative amount of nitrogen in urea and in ammonia, in the earlier experiments these constituents were alone specially investigated, but in the later experiments other constituents were also dealt with.

II.—Method of Experiment.

Female dogs of about 15 to 20 kilos. were used for the experiments. Catheterisation was not used in collecting the urine. This was done by keeping the dog in a galvanised iron cage with a sloping bottom, under which the urine, after passing through a rough filter of glass-wool, was collected in a glass vessel containing a little dilute hydrochloric acid to prevent decomposition. The dog was fed on porridge and milk, which produced firm fæces, which were at once removed from the cage, and with which the urine was never contaminated. (For a comparison of the analysis of urine collected thus and by catheterisation, see *Jour. of Physiol.*, xxv., p. 445, 1900.) The diet generally consisted of:—

Oatmeal = 120 grm.
Water = 500 c.c.

made into porridge by boiling for twenty minutes.

Milk = 500 c.c.

When nitrogenous equilibrium was established, the chloroform was administered:—

A. In the first series of experiments, by the respiratory passages, with the addition of 10 per cent. alcohol, following Schäfer's recommendation for decreasing the toxic effect upon the heart (*Trans. Roy. Soc. Edin.*, vol. xli., p. 311, 1904).

B. In the second set of experiments, by the stomach, dissolved in oil.

C. In the third set of experiments, by hypodermic injection.

In some of the earlier experiments the dog fasted on the day on which the chloroform was administered, the effect of a one-day fast being investigated a few days before the chloroform was given.

III.—Methods of Analysis.

The urine was collected at 10 each morning. It was measured, the reaction and specific gravity taken, and either the whole or a part diluted to a convenient volume. When a deposit was present, it was collected in a Y-glass and examined microscopically.

The Total Nitrogen was determined by Kjeldahl's method.

The "Urea" Nitrogen was determined by Bohland's or by Mörner and Sjöqvist's method, or by both. A useful comparison of these two methods is thus afforded. (See *Jour. of Physiol.*, vol. xxxiii., p. 6, 1905.)

The Ammonia Nitrogen was determined by Schloessing's method.

The Creatinin Nitrogen, when investigated, was determined by Folin's method.

The Uric Acid Nitrogen in the early experiments was determined by Hopkins' method, and in the later experiments (XI. and XII.) by Folin's method.

The Oxidised and Unoxidised Sulphur were determined by the method described in *Jour. of Physiol.*, vol. xxiv., p. 335, 1899.

Most of the determinations of nitrogen were carried out under my supervision by Mr Alfred Patterson, Chemical Attendant in the Royal College of Physicians Laboratory. The sulphur determinations were made by Miss Jean Robertson, Chemical Assistant in the Laboratory.

IV.—Results.

Series I.—Chloroform by the Respiratory Passages.

The chloroform was administered upon cotton wadding placed in a mask. Since several determinations have now been recorded of the chloroform in the blood during full anæsthesia, showing that it amounts to something not far short of 50 mg. per 100 c.c. (Gréhant and Quinquand, *C.R.*, 1883: Nicloux, *C.R. Soc. biol.*, t. lx., p. 244; Buckmaster and Gardner, *Proc. Roy. Soc.*, vol. lxxix., 1907), it was not considered necessary to dose the amount given. Generally in two hours about 80 c.c. were used.

Experiment I.—Retriever Bitch. Weight, 16 kilos.

Table I.

Day.	Urine in c.c.	Sp. G.	Total Nitrogen.	Urea Nitrogen.		NH_3 Nitrogen.		
				Bohl.*	M. & S.†			
1	880	1013	6·22	5·32	...	0·579		
2	910	1013	4·96	...	...	0·403		
3	620	1013	4·12	...	...	0·299		
4	690	1014	4·06	3·39	...	0·274		
5	220	1025	2·77	2·41	2·27	0·126	Fast.	
6	640	1010	3·95	3·33	3·11	0·252		
7	570	1013	3·30	2·86	2·66	0·224		
8	770	1018	6·94	5·63	5·68	0·512		
9	690	1015	5·48	4·68	4·56	0·397	Fast.	Chloroform 1 hour.
10	770	1014	4·87	4·26	4·01	0·229		
11	690	1012	4·09	3·50	3·33	0·319		

Weight, 15 kilos.

No protein and no reduction of Fehling's solution.

* Bohl. = Bohland's phospho-tungstic acid method.

† M. & S. = Morner and Sjoqvist's method.

Table II.

Day.	Percentage of Total Nitrogen.				
	Urea.		NH_3.	Not in these.	
	Bohl.	M. & S.			
1	86	...	9·3	...	
2	...	...	8·5	...	
3	...	...	7·2	...	
4	86	...	6·7	...	
5	87	82	4·5	13·5	Fast.
6	84	78	6·2	15·8	
7	85	81	6·7	12·3	
8	81	82	7·3	10·7	
9	88	84	7·3	8·7	Fast. Chloroform 1 hour.
10	87	83	4·6	12·4	
11	85	81	7·8	11·2	

Experiment II.—Retriever Bitch. Weight, 16 kilos.

Table III.

Day.	Urine in c.c.	Sp. G.	Total Nitrogen.	Urea Nitrogen. M. & S.	NH_3 Nitrogen.	
1	700	1013	4·54	3·56	0·476	
2	Lost	...	...	...	...	
3	720	1012	3·70	...	0·411	Fast. Chloroform 2 hours in morning and 1 hour in afternoon.
4	300	1025	4·20	3·43	0·304	
5	260	1040	9·41	8·09	0·420	
6	680	1016	6·66	5·46	0·515	
7	570	1017	5·71	4·45	0·420	
8	480	1015	4·28	3·23	0·268	
9	770	1012	4·93	3·89	0·341	
10	680	...	...	...	...	

Weight, 16·6 kilos.

On the second day after chloroform there was a distinct trace of protein in the urine—by Esbach's tube 0·04 per cent. On the third day this increased to 0·1 per cent. It disappeared on the sixth day. On the third day there was a copious deposit of granular cells like renal epithelium, but no tube-casts. These cells were present till the sixth day. The urine was very dark for three days. No reduction of Fehling's solution.

Table IV.

Day.	Percentage of Total Nitrogen.		Not in these.	
	Urea. M. & S.	NH_3.		
1	78	10·4	11·6	
2	...	...	...	
3	...	11·1	...	
4	83	7·2	9·8	Fast. Chloroform 3 hours.
5	86	4·4	9·6	
6	82	7·7	10·3	
7	79	7·5	13·5	
8	79	6·4	14·6	
9	79	6·9	14·1	
10	...	...	...	

Experiment III.—Retriever Bitch. Weight, 14·7 kilos.

Table V.

Day.	Urine in c.c.	Sp. G.	Total Nitrogen.	Urea Nitrogen. Bohl.	NH_3 Nitrogen.	
1	450	1018	4·56	...	0·515	
2	560	1013	4·00	3·14	0·434	
3	520	1014	3·78	2·87	0·403	
4	320	1025	3·84	3·28	0·218	Fast. Chloroform 2 hours.
5	220	1032	5·49	4·76	0·330	
6	570	1015	3·98	3·44	0·344	
7	550	1012	3·08	2·83	0·232	
8	570	1014	3·08	2·49	0·145	
9	560	1015	...	...	...	
10	710	1013	2·58	1·90	0·254	
11	630	1015	...	...	...	

Dog weighed 13·6 kilos.

A trace of protein appeared on the fourth day after chloroform, and was more marked on the sixth day. On the fourth day there was a deposit of granular cells, but no tube-casts. The urine gave a very slight reduction of Fehling's solution on the day after chloroform.

Table VI.

Day.	Percentage of Total Nitrogen.		Not in these.	
	Urea. Bohl.	NH_3.		
1	...	11·3	11·7	
2	78	10·3	11·7	
3	75	10·6	14·4	
4	85	5·6	9·4	Fast. Chloroform 2 hours.
5	86	6·0	8·0	
6	86	8·5	5·5	
7	91	7·5	8·5	
8	80	4·6		
9	...	...	...	
10	75	9·6	5·6	

Experiment IV.—Retriever Bitch. Weight, 14·7 kilos.

Table VII.

Day.	Urine in c.c.	Sp. G.	Total Nitrogen.	Urea Nitrogen.		NH_3 Nitrogen.	
				Bohl.	M. & S.		
1	630	1015	5·38	4·51	4·51	0·375	
2	310	1031	5·24	4·37	4·34	0·350	Chloroform 2 hours.
3	380	1028	8·99	7·59	7·70	0·616	
4	570	1020	6·99	5·74	5·63	0·444	
5	735	1013	5·77	4·68	4·73	0·406	
6	950	1013	6·27	5·12	5·12	0·434	
7	520	1015	4·22	3·47	3·50	0·246	
8	630	1015	4·79	3·95	3·89	0·330	
9	975	1011	...	...	...	...	

Dog weighed 12 kilos.

No protein. No reduction of Fehling's solution.

Table VIII.

Day.	Percentage of Total Nitrogen.				
	Urea.		NH_3.	Not in these.	
	Bohl.	M. & S.			
1	83·5	83	6·9	10·1	
2	83·3	83	6·6	10·4	Chloroform 2 hours.
3	83	84	6·9	9·1	
4	82	80·5	6·3	13·2	
5	81	82	7·0	11·0	
6	81·6	81·6	6·9	11·5	
7	82	83	5·8	11·2	
8	80	81	6·8	12·8	

Experiment V.—Retriever Bitch. Weight, 12·8 kilos.

A weighed quantity of plasmon was added to the diet.

Table IX.

Day.	Urine in c.c.	Sp. G.	Total Nitrogen.	Urea Nitrogen.		
				Bohl.	M. & S.	
1	620	1022	9·04	7·34	7·34	
2	50	1016	0·89	0·71	...	Fast.
3	410	1031	9·18	7·70	7·81	
4	740	1015	7·73	6·78	6·64	
5	140	1047	4·57	4·10	4·08	Fast. Ether for 2 hours.
6	660	1022	9·72	8·60	8·48	
7	620	1022	9·52	8·32	8·26	
8	300	1037	8·15	7·36	7·25	Fast. Chloroform for 2 hours.
9	450	1031	10·9	9·80	9·83	
10	560	1020	7·84	7·08	6·97	
11	530	1023	10·11	9·16	9·04	
12	430	1025	8·57	7·59	7·39	

Dog weighed 10 kilos.

Trace of protein on day after ether. Marked reduction of Fehling's solution on day of chloroform. Trace of protein on day after chloroform.

Table X.

Day.	Percentage of Total Nitrogen in Urea.		
	Bohl.	M. & S.	
1	81	81	
2	79	...	Fast.
3	83	85	
4	87	86	
5	89	89	Fast. Ether for 2 hours.
6	88	87	
7	87	87	
8	90	89	Fast. Chloroform for 2 hours.
9	90	90	
10	90	89	
11	90	89	
12	87	86	

Table XI.

Day.	Total Sulphur.	Sulphur Oxidised.	Oxidised per cent. of Total Sulphur.	
1	...	...	...	
2	...	...	...	
3	1·34	0·941	70	
4	1·16	0·803	69	
5	0·80	0·547	68	Ether.
6	1·50	1·11	74	
7	2·17	1·37	63	
8	...	...	...	Chloroform.
9	1·83	1·20	66	
10	1·72	...	...	
11	2·03	1·38	68	
12	1·39	...	...	

Experiment VI.—Setter Bitch. Weight, 15·1 kilos.

Food—oatmeal, 100 grm.; water, 500 c.c.; milk, 500 c.c.

Table XII.

Day.	Urine in c.c.	Sp. G.	Total Nitrogen.	Urea Nitrogen. M. & S.	NH_3 Nitrogen.	Creatinin Nitrogen.	
1	970	1012	5·68	...	...	0·15	
2	860	1013	5·94	...	...	0·15	
3	160	1032	2·74	...	...	0·04	
4	870	1012	5·18	4·34	0·24	0·13	{ Chloroform
5	420	1020	4·41	3·74	0·22	0·10	{ for 3 hours.
6	490	1025	8·99	7·64	0·54	0·13	
7	660	1014	7·84	6·40	0·46	0·12	
8	620	1016	6·50	5·40	0·34	0·13	
9	550	1016	5·33	4·41	0·38	0·10	
10	575	1010	3·54	3·04	0·21	0·06	
11	1100	1015	8·61	7·44	0·60	0·13	
12	225	1025	3·54	...	...	0·06	

Dog weighed 12·6 kilos.

On the day after chloroform the urine was dark in colour, and remained so for four days. No proteins; no reduction of Fehling's solution; no bile-pigments.

Table XIII.

Day.	Percentage of Total Nitrogen.		Creatinin.	Nitrogen not in these.	
	Urea. M. & S.	NH_3.			
1	...	...	2·6	...	
2	...	...	2·5	...	
3	...	...	1·4	...	
4	83	4·6	2·0	10·4	
5	85	5·0	2·4	7·6	Chloroform for 3 hours.
6	85	6·0	1·4	7·6	
7	81	5·9	1·5	10·9	
8	83	5·2	1·9	10·0	
9	82	7·0	1·97	9·0	
10	85	6·0	1·9	7·0	
11	86	7·0	1·4	5·6	
12	...	...	1·6	...	

Table XIV.

Day.	Total Sulphur.	Sulphur Oxidised.	Oxidised per cent. of Total Sulphur.	
1	0·89	0·53	66	
2	1·17	0·72	61	
3	0·56	0·27	49 } 63	
4	0·95	0·74	78 } 63	
5	1·05	0·76	72	Chloroform.
6	1·13	0·76	67	
7	1·19	0·81	68	
8	1·15	0·83	72	
9	1·03	0·61	60	
10	0·61	0·40	66	
11	1·94	1·60	82 } 62	
12	0·81	0·34	42 } 62	

The results of these experiments may be considered under the following heads:—

1. *Amount of Water excreted.*—The general result shows that chloroform tends to diminish the secretion of urine on the day on which it is administered (see Thompson, *loc. cit.*).

2. *Total Nitrogen.*—In these experiments the increased excretion of nitrogen, *i.e.* the increased protein katabolism, already recorded by Strassmann, Vidal, and others (*loc. cit.*), is shown in Experiments II., IV., and VI., but not in I., III., and V. (see Table I.).

Table XV.

Total Nitrogen.

	Before.	After.
Experiment I.	Average of 4 days, 4·24	Average of 3 days, 4·81
„ II.	„ 2 „ 4·06	„ „ 7·26
„ III.	„ 3 „ 4·11	„ „ 4·11
„ IV.	„ 1 „ 5·38	„ „ 7·60
„ V.	„ 2 „ 9·62	„ „ 9·60
„ VI.	„ 4 „ 4·91	„ „ 7·77

3. *Urea Nitrogen.*—Without entering upon a discussion of the question of how far the nitrogen determined by Bohland's and by Mörner's and Sjöqvist's methods actually represents the nitrogen elaborated into urea (see *Jour. of Phys.*, xxxii., 1, 1905), these experiments show that, in the absence of amino acids, both give a fair approximation to the true result, and that both methods are of value for comparative results.

The results of these experiments may be tabulated:—

TABLE XVI.

Urea Nitrogen.

Exp.	Bohland's. Before.	Bohland's. After.	Bohland's. Per cent. Rise.	Morner's. Before.	Morner's. After.	Morner's. Per cent. Rise.
1	84 85	88 87	4	78 81	84 83	6
2	...	...	...	78	86	10
3	78 75	86 86	10	74 73·5	86 87	10
4	83·5	84 82	0	83	84 80·5	0
5	88 87	90 90	3	87 87	90 89	3
6	...	...	...	83	85 81	·01

The effect of the administration of chloroform to the extent to maintain full anæsthesia for periods of from one to three hours in the dog is generally, although not always, to cause a rise in the proportion of nitrogen in the form of urea.

4. *Ammonia Nitrogen.*—In Experiments I., II., and III., there was a distinct fall in the proportion of nitrogen in ammonia; in IV. and VI., where the rise in urea nitrogen was absent, there was no change. In V. it was not determined.

5. *Creatinin Nitrogen.*—In the one experiment in which this was investigated (Experiment VI.) there was unfortunately no change in the distribution of the "urea" nitrogen. No very marked change in the amount of creatinin nitrogen could be determined, but it roughly follows the excretion of total nitrogen. There is no distinct fall in the proportion of creatinin nitrogen.

6. *The Purin and Uric Acid Nitrogen* were not investigated in this series.

7. *The Nitrogen not in Urea and Ammonia* showed a fall in Experiments I., II., and III., the experiments in which the urea nitrogen rose, and no marked change in IV. and VI.

8. *Sulphur.*—This was determined only in Experiments V. and VI., where the disturbance in the proportion of urea nitrogen was trivial or absent. The total sulphur ran fairly parallel with the total nitrogen, although the usual divergences were manifest.

The Oxidised Sulphur, both as regards total amount and its proportion to the total sulphur, showed no variation ascribable to the influence of

chloroform. A curious tendency for a day of low proportionate excretion to be followed or preceded by a day of high excretion was observed.

In these experiments protein was present in the urine once (Experiment II.) in appreciable amount, and twice (Experiments III. and IV.) in mere traces. In Experiment II. there was a deposit of fatty cells resembling those of the kidney, but not forming tube-casts; and a few similar cells were present in Experiment III.

No reduction of Fehling's solution was obtained except in Experiment III., when a very slight reduction was noted on the day after the administration of chloroform. The reduction was so slight that no attempt was made to determine if it was due to sugar.

In Experiment V. the administration of ether for two hours, although it was followed by a slight albuminuria, caused no marked change in the protein metabolism.

Series II.—Chloroform by the Stomach.

In the next two experiments 20 to 25 c.c. of chloroform dissolved in 100 c.c. of olive oil were administered by the stomach-tube, and as sickness was to be expected, food was withheld on the day of administration, sometimes entirely, sometimes merely till late in the evening. The dog was on both occasions somewhat excited, but showed no signs of anæsthesia.

Experiment VII.—Setter Bitch. Weight, 18 kilos.

Table XVII.

Day.	Urine in c.c.	Sp. G.	Total Nitrogen.	Urea Nitrogen. M. & S.	Uric Acid Nitrogen.	Creatinin Nitrogen.	NH_3 Nitrogen.
1	570	1016	} 5·99	5·28	0·079	...	} 0·128
2	910	1014	} 5·91	5·28	0·091	0·124	} 0·128
*3	410	1016	3·42	2·83	0·049	0·079	0·123
†4	360	1036	6·42	5·28	0·018	0·126	0·44
5	945	1018	12·56	10·24	0·189	0·236	0·96
6	675	1016	6·01	4·46	0·135	0·145	0·527
7	360	1019	4·54	3·50	0·032	0·149	0·316
8	830	1014	6·31	5·20	0·074	0·150	0·594
9	360	1013	3·29	2·70	0·050	0·044	0·134
10	780	1012	5·38	4·54	0·085	0·079	0·260
11	560	1017	5·29	4·10	...	...	...
12	920	1014	...	...	...	...	...

Dog weighed 15 kilos.

On third day dog fasted, and 20 c.c. chloroform in 75 c.c. olive oil given. Dog vomited.*

On fourth day dog fasted, and 20 c.c. chloroform in 75 c.c. olive oil given at 1 p.m. Dog took food at night.†

Next day urine dark. No protein. No reduction of Fehling's solution. Two days later a trace of protein and a large deposit of fatty cells. Next day no protein reaction.

Table XVIII.

Day.	Percentage of Total Nitrogen.				Nitrogen not in these.
	M. & S.	Uric Acid.	Creatinin.	NH_3.	
1	89	1·4	...	2·1	...
2	89	1·4	...	2·1	...
*3	82	1·4	2·0	7·0	7·6
†4	82	2·8	1·9	6·0	7·3
5	82	1·5	1·8	7·6	7·1
6	74	{ 2·2 } 1·5	2·4	8·6	11·9
7	77	{ 0·7 }	2·3	6·9	13·1
8	82	1·1	2·3	9·4	5·2
9	84	1·5	1·3	3·8	9·4
10	83	1·5	1·4	4·8	9·3
11	...	....	...	...	...
12	...	...	...	...	...

Table XIX.

Sulphur.

Day.	Total.	Oxidised.	Oxidised per cent. of Total Sulphur.
1	0·46	0·30	65
2	1·19	0·79	66
*3	0·81	0·48	59
†4	1·20	0·70	58
5	2·40	1·36	56
6	1·20	0·81	67
7	0·74	0·50	67
8	1·17	0·65	55
9	0·44	0·25	56
10	0·81	0·54	66
11	0·92	0·60	65
12	...	...	...

Experiment VIII.—Same Dog. Weight, 15·4 kilos.

Table XX.

Day.	Urine in c.c.	Sp. G.	Total Nitrogen.	Urea Nitrogen. M. & S.	Uric Acid Nitrogen.	Creatinin Nitrogen.	NH_3 Nitrogen.
1	1080	1011	6·06	5·01	0·05	0·119	0·400
2	565	1012	6·06	5·01	0·05	0·119	0·400
*3	370	1015	3·19	2·77	0·007	0·085	0·249
†4	440	1036	10·30	8·46	0·026	0·149	0·638
5	425	1020	5·46	4·17	0·017	0·100	0·557
6	470	1022	5·21	4·12	0·101	0·14	0·386
7	970	1012	5·77	4·34	0·174	0·12	0·408
8	1020	1011	4·33	3·39	0·091	0·086	0·413
9	670	1013	4·73	3·84	0·080	0·12	0·492
10	490	1011	3·02	2·43	0·039	0·069	0·199
11	655	1012	3·42	2·88	0·052	...	0·182

Dog weighed 14 kilos.

On third day, 25 c.c. chloroform in oil at 1 p.m. Dog vomited.*

On fourth day, 25 c.c. chloroform in oil at 11 a.m. Took food at night.†

On day after urine dark. Gmelin's reaction. Trace of protein. No reduction of Fehling's solution. Deposit of fatty renal cells. Gmelin's test positive for three days. Protein disappeared on second day, but fatty cells and tube-casts present. Five days after chloroform the urine seemed normal.

Table XXI.

Day.	Percentage of Total Nitrogen.				Nitrogen not in these.	
	M. & S.	Uric Acid.	Creatinin.	NH_3.		
1	82	0·8	1·9	5·4	9·9	
2	82	0·8	1·9	5·4	9·9	
3	83	0·2	2·6	7·8	6·4	Chloroform.
4	82	0·2	1·4	6·2	10·2	,,
5	76	0·3	1·8	10·2	11·7	
6	71	1·9	2·6	7·4	17·1	
7	75	3·0	2·0	7·0	13·0	
8	78	2·1	2·0	9·5	8·4	
9	81	1·7	2·5	10·4	4·4	
10	80	1·0	2·3	6·6	10·1	
11	82	1·5	...	5·3	...	

1. *Total Nitrogen.*—These experiments show the very marked effects of chloroform administered by the stomach upon the protein katabolism. In both, a very markedly increased excretion of total nitrogen followed the administration of the substance.

2. *Urea Nitrogen.*—A very marked fall in the proportion of urea nitrogen, as determined by Mörner and Sjöqvist's method, is manifest in each experiment, reaching its maximum on the third day. In Experiment VII. it amounts to 17 per cent., and in Experiment VIII. to 13 per cent. It is to be regretted that the conformity of the results obtained by the two methods of determining the urea nitrogen in the previous series of experiments decided me to employ one only in the present set.

3. *Ammonia Nitrogen.*—A rise in the proportion of ammonia nitrogen accompanies the fall in urea nitrogen, but the maximum of the one does not always occur on the same day as the minimum of the other (figs. 1 and 2, p. 493). In both experiments the maximum proportion of ammonia nitrogen is reached two or three days after the minimum proportion of urea nitrogen.

4. *Creatinin Nitrogen.*—The total excretion of creatinin nitrogen in these experiments follows fairly closely the excretion of total nitrogen. Hence the *proportionate* excretion does not undergo very marked variations, but in both experiments the rise in creatinin nitrogen is not proportionate to the rise in total nitrogen on the day after the administration, and hence the proportion falls somewhat.

5. *Uric Acid Nitrogen.*—In both experiments there is an increase in the total amount of uric acid nitrogen excreted, but this occurs earlier, on the day following the chloroform, in Experiment VII., and later, three or four days after, in Experiment VIII. In Experiment VII. the proportionate increase appears on the day after the administration, and in Experiment VIII. not until the third day and after.

6. *The Nitrogen not in Urea and Ammonia,* and the nitrogen not in urea, ammonia, creatinin, and uric acid, show a rise after the administration of chloroform.

7. *Sulphur.*—The excretion of sulphur in Experiment VII. runs parallel with the excretion of total nitrogen. The proportion of oxidised sulphur is decreased after the administration of chloroform, but is not lowest on the day of the lowest proportion of urea nitrogen.

In both experiments, proteins, granular cells, and tube-casts were present in the urine. There was no reduction of Fehling's solution. In Experiment VIII. bile-pigments appeared in the urine, and the animal was slightly jaundiced.

Series III.—Hypodermic Administration.

Two experiments were performed upon dogs of 18 and 15 kilos., 5 c.c. of chloroform being administered in Experiment IX. on two successive days, and in Experiment X. twice on the same day. The animals showed no sign of intoxication, but in Experiment X. the dog took only half its food on the day of administration and the next day, and the milk only on the following day.

EXPERIMENT IX.—Same Dog. Weight, 18 kilos.

TABLE XXII.

Day.	Urine in c.c.	Sp. G.	Total Nitrogen.	Urea Nitrogen. M. & S.	Uric Acid Nitrogen.	Creatinin Nitrogen.	NH_3 Nitrogen.
1	335	1028	5·26	4·62	...	...	...
2	640	1018	6·92	6·36	...	0·22	...
3	745	1015	6·55	5·68	·007	0·25	0·238
*4	910	1012	6·24	5·24	·000	...	0·439
†5	165	1026	6·03	4·98	·034	0·24	0·369
6	690	1022	6·03	4·98	·034	0·24	0·369
7	610	1017	5·71	4·68	·048	0·13	0·277
8	740	1014	3·64	2·60	·059	0·15	0·305
9	515	1016	5·29	4·23	·046	0·20	0·420
10	680	1017	6·38	5·40	·040	0·24	0·313
11	580	1015	4·68	4·06	·029	0·10	0·288
12	775	1011	4·16	3·50	·023	0·14	...
13	500	1010	2·83	...	...	0·09	...
14	730	1017	5·72	...	...	0·15	...

Dog weighed 15·8 kilos.

On fourth day, 5 c.c. chloroform.*

On fifth day, 5 c.c. chloroform.†

From second day of chloroform to third day after, slight reduction of Fehling's solution on cooling.

From next day to fourth after, protein present; on third day, deposit of fatty cells.

Table XXIII.

Day.	Percentage of Total Nitrogen.				Nitrogen not in these.
	M. & S.	Uric Acid.	Creatinin.	NH_3.	
1	87	...	...	...	...
2	91	...	3·2	...	...
3	86	0·1	3·3	3·6	6·5
*4	84	0·0	...	6·8	...
†5	82	0·5	3·9	6·1	7·5
6	82	0·5	3·9	6·1	7·5
7	81	0·8	2·3	4·8	11·0
8	72	1·6	4·2	8·3	13·9
9	80	0·8	3·7	7·9	7·6
10	84	0·6	3·9	4·9	6·6
11	87	0·6	2·1	6·8	3·5
12	84	0·5	3·3	...	...
13	...	...	2·6	...	...
14	...	...	...	...	...

Table XXIV.

Sulphur.

Day.	Total.	Oxidised.	Oxidised per cent. of Total Sulphur.
1	0·782	0·624	79
2	0·927	0·734	79
3	0·940	0·741	79
*4	0·940	0·628	66
†5	0·940	0·628	66
6	1·08	0·721	66
7	0·968	0·583	60
8	0·940	0·618	66
9	0·707	0·466	66
10	0·796	0·549	68
11	0·755	0·556	73
12	...	...	...
13	...	...	...
14	...	...	...

Experiment X.—Collie Bitch. Weight, 15·4 kilos.

Table XXV.

Day.	Urine in c.c.	Sp. G.	Total Nitrogen.	Urea Nitrogen. M. & S.	Uric Acid Nitrogen.	Creatinin Nitrogen.	NH_3 Nitrogen.
1	400	1012	2·26	1·84	...	0·08	0·076
2	880	1016	3·98	3·28	...	0·16	0·347
3	460	1010	2·20	1·39	...	0·09	0·140
9	530	1007	1·51	1·38	0·00	0·05	0·066
10	640	1010	3·88	2·45	0·00	0·10	0·238
*11	770	1012	5·56	...	...	0·18	0·409
12	275	1017	4·65	3·91	0·018	0·11	0·608
13	550	1016	4·65	3·91	0·018	0·11	...
14	...	...	...	...	...	...	...
15	610	1017	6·36	5·24	0·073	0·20	0·456
16	730	1010	3·75	2·96	0·051	0·11	0·419
17	550	1010	3·61	2·11	0·022	0·08	0·148
18	700	1010	4·21	3·43	0·028	0·12	0·329

Dog weighed 14·7 kilos.

On eleventh day, 5 c.c. chloroform at 11, and again at 5.*

From the day of chloroform to fourth day after only part of food was taken.

On second day after, trace of protein, present till eighteenth day; on fourth day, 0·07 per cent. by Esbach's method.

Slight reduction of Fehling's solution from second to fourth day after.

Table XXVI.

Day.	Percentage of Total Nitrogen.				Nitrogen not in these.
	M. & S.	Uric Acid.	Creatinin.	NH_3.	
1	83	...	3·9	3·3	...
2		...	4·0	8·6	...
3		...	4·0	6·3	...
9	84	0·0	3·3	4·4	7
10		0·0	3·2	7·1	
*11	...	...	3·2	7·4	...
12	83	0·4	2·4	6·5	8
13	...	0·4	...	...	...
14	...	...	...	...	...
15	82	1·1	3·1	7·1	7
16	79	1·3	2·9	11·3	5·5
17	81	0·6	3·0	5·8	9·6
18	81	0·6	2·8	7·9	7·7

1. *Total Nitrogen.*—These two experiments show no definite change in the total nitrogen excreted, although in the second experiment a possible rise is masked by the fact that the dog did not take all its food.

2. *Urea Nitrogen.*—In Experiment IX. there is a distinct fall in the proportion of urea nitrogen during three days after the administration of chloroform, reaching its maximum, 18 per cent., on the third day. In Experiment X. the fall is slight, less than 5 per cent., and is delayed till the fifth day.

3. *Ammonia Nitrogen.*—In Experiment IX., on the third day, the day of the most marked fall in urea nitrogen, there is a rise in the proportion of ammonia nitrogen, and the same thing occurs in Experiment X. on the fifth day.

4. *Creatinin Nitrogen.*—In neither experiment is there any marked change in the proportion of creatinin nitrogen. The total amount excreted varies fairly directly with the excretion of total nitrogen.

5. *Uric Acid Nitrogen.*—In Experiment IX. the *proportion* of uric acid nitrogen is markedly raised, especially on the day of the lowest excretion of urea nitrogen. In Experiment X. the change is not evident. In both experiments there is a distinct increase in the *total excretion* of uric acid nitrogen; in the first, upon the third day after the administration of chloroform, and in the second, upon the first and second days after.

6. *Nitrogen not in these Combinations.*—In the first experiment there is a distinct rise upon the second and third day after the chloroform. In the second experiment a very doubtful rise occurs upon the sixth day.

7. *Sulphur.*—The sulphur was estimated in the first experiment only. The proportion of oxidised sulphur shows a marked decrease, beginning on the day after the chloroform, and reaching its maximum on the third day after—the day of the greatest fall in urea nitrogen.

In both these experiments proteins were present in the urine, and in the first fatty cells resembling renal epithelium were seen. They were unfortunately not examined for in the second experiment. In neither experiment was there any marked reduction of Fehling's solution.

V.—Consideration of Results.

These experiments show that in the dog chloroform, *when given by the respiratory passages*, for two or three hours has either no effect upon the nitrogenous metabolism or increases the protein disintegration, as already demonstrated by previous investigators. The effect upon the nitrogen in urea was either to leave it unaffected or *to increase its proportion*, while, at the same time, the nitrogen in ammonia was either unchanged or *decreased* when the urea was increased. These results suggest that the hepatic metabolism, by which ammonia compounds are changed to urea, is stimulated—an effect very similar to that produced by the administration of a protein diet (*Jour. of Phys.*, vol. xxv., p. 443, 1900)—and that this stimulation, in these experiments at least, is not followed by any after-depression.

The toxic action of the drug upon the kidneys is manifested by the presence of proteins and of renal epithelium in the urine, most markedly in Experiment II., where the animal was anæsthetised for two hours, and again for one hour.

When chloroform is given *by the stomach* the effect is very different. The protein disintegration, as indicated by the excretion of nitrogen, is markedly increased, and this increase is best marked on the day of, or the day after, the administration of the drug. At this time the disturbance in the proportion of nitrogen in the various compounds is not very pronounced, and the presence of protein in the urine is not so distinctly marked as it afterwards becomes. There is apparently a period of simple increase of the protein disintegration.

After two or three days there occurs a very marked fall in the proportion of nitrogen in urea, with a rise in the proportion of nitrogen in ammonia—a rise which, however, is not inversely proportionate to the fall in urea nitrogen, and which is somewhat delayed (figs. 1 and 2). Along with this there is a rise in the proportion of uric acid nitrogen, which apparently may precede (Experiment VII.) or succeed (Experiment VIII.) the most marked fall in the urea nitrogen. The proportion of nitrogen not in these analysed compounds increases concomitantly with the fall in the urea, and previously to the rise in the ammonia. In both the experiments the rise was a very marked one, corresponding to something like 6 per cent. of the total nitrogen. Since the urea nitrogen was determined by the method of Mörner and Sjöqvist, the amino acids were precipitated, and not estimated with the urea, and hence almost certainly this increase in the undetermined nitrogen is due to the appearance of amino acids. It is to be regretted that, since the previous examination of the urine after the

administration of chloroform by the respiratory passages had shown so close a correspondence between the results of the methods of Bohland and of Mörner and Sjöqvist, the former method was abandoned in the present series of analyses, for a divergence between these would have more definitely proved that the undetermined nitrogen is really in amino acids. That the rise in the proportion of undetermined nitrogen was not due to the appearance of protein is indicated, first, by the fact that in Experiment VII. the rise occurred before the appearance of the protein, and, second, by

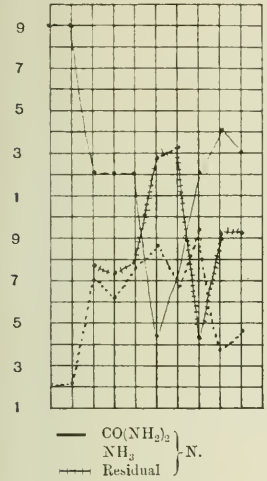


Fig. 1.—Experiment VII.

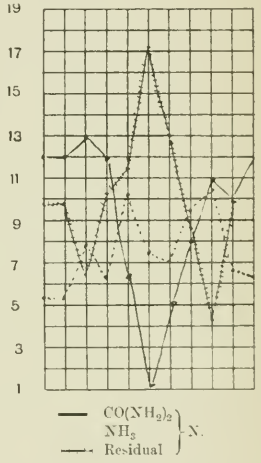


Fig. 2.—Experiment VIII.

the fact that the amount of protein was insufficient to account for the nitrogen thus excreted.

The proportion of unoxidised sulphur to the total sulphur (Experiment VII.) shows, like the nitrogen in urea, a distinct fall, curiously interrupted by a rise on the two days on which the proportion of urea nitrogen was lowest. I am unable to explain this.

The whole series of changes is such as would be produced by a direct toxic action on the liver, similar to that produced by the administration of alcohol and sulphonal (*Jour. of Phys.*, xxvi., p. 166, 1901). The effect is not immediate, but is nevertheless the result of the poisoning of the cells. This is clearly shown by the histological changes produced in the liver cells by giving chloroform by the mouth, already described by Doyon and

now being investigated by Dr Clark. The effect upon the kidney, as indicated by the presence of protein and the appearance of renal epithelium in the urine, is much more marked when chloroform is given by the mouth than when administered by the respiratory passages. Zweifel (*Berl. klin. Wochensch.*, 1874, p. 245) and Pohl (*Arch. f. exp. Path. u. Pharmac.*, Bd. xxviii., p. 251) found traces of chloroform in the urine, and Nicloux (*Jour. de Pharm. et Chim.*, 24 [6], p. 64) has found that the amount after administration by the respiratory passages is very small. But the fact that protein appeared in the urine in certain of my experiments in which it was given in this way without exerting a true toxic action on the liver, and the very frequent occurrence of transient albuminuria described by many surgeons, seems to indicate that its effect on the kidney is direct.

The question naturally arises of how far these differences in the effect of chloroform given by the respiratory passages and by the mouth are merely a result of difference of dose. The amount of chloroform in the blood necessary to produce anæsthesia has now been investigated by several observers—Tissot (*C.R. Soc. biol.*, 1906, p. 198), Nicloux (*C.R. Soc. biol.*, t. lx., p. 144), Buckmaster (*Proc. Roy. Soc.*, vol. lxxix., 1907)—and the general result is to indicate that, to produce the state, something like 30 to 50 mg. per 100 c.c. must be present in the blood of the dog or cat. After the administration is stopped, the amount rapidly falls, and the elimination is practically complete in about three hours (see succeeding paper).

So far no determinations of the amount of chloroform in the blood after it has been administered by the mouth have been recorded, but a series of experiments carried out by Miss D. Lindsay upon the blood of rabbits after the administration of chloroform in oil, nearly equivalent to that given by me to the dogs experimented upon, shows that the percentage amount never rises so high as during administration by the lungs, and that the drug persists in the blood for a much longer period—about six hours.

The greater effect upon the metabolism of chloroform when administered by the mouth is not the result of a larger dose, but must be due rather to the direct influence upon the liver, and to the more prolonged action.

In rabbits, I have found that the administration of 1 c.c. by the mouth, which leads to the appearance of at most between 20 and 30 mg. per 100 of $CHCl_3$ in the blood, is infinitely more toxic than a prolonged administration by the lungs. Of 12 rabbits, no less than 11 showed albuminuria, while 7 died, and 2 which appeared to be dying were killed.

How far the fatal result is due to the injury to the kidneys, how far to the toxic action on the liver, and how far to the more general injury to the tissues produced, we have no data to enable us to determine. The

appearance of acetone and diacetic acid in the urine shown by various investigators is probably a mere expression of this disturbed metabolism, whereby the β oxybutyric acid set free does not undergo its normal katabolism, but is converted into acetone. It is probably secondary to the hepatic change, and is an accompaniment, not the cause, of the death of the animal.

A study of the effects of *hypodermic* administration of the drug throws some light upon its mode of action. With a dose in two portions of 5 c.c.

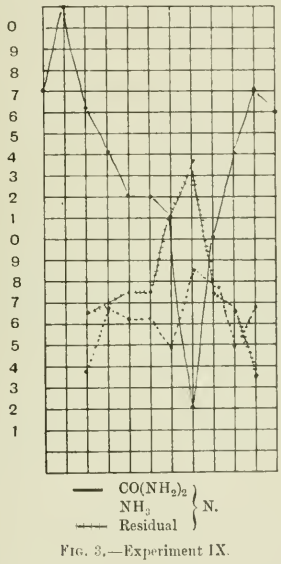


Fig. 3.—Experiment IX.

there was no definite change in the excretion of nitrogen: but in Experiment IX. there was a sharp fall in the urea nitrogen, with a corresponding rise in the amino acid nitrogen, somewhat preceding a rise in the ammonia nitrogen (fig. 3). In Experiment X. the changes were less marked, but in the same direction.

Given hypodermically, chloroform, then, acted in the same manner as when given by the mouth, but to a lesser degree. How far this lesser degree is due to the smaller dose administered it is difficult to say. Miss Lindsay's observations on rabbits seem to show that the administration of 1 c.c. of chloroform by the mouth leads to the appearance of a higher

percentage of chloroform in the arterial blood than does the administration of the same amount hypodermically; and hence dogs which got two doses of 25 c.c. of chloroform by the mouth may have had a higher proportion of the drug in the circulating blood than those which had two doses of 5 c.c. hypodermically. The respect in which the hypodermic administration resembles the administration by the mouth is in the persistence of the drug.

The hypodermic administration of chloroform to rabbits appears to be almost as fatal as the administration by the mouth, and the appearance of proteins and tube-casts in the urine is almost as frequent. Dr Clark also finds that the changes in the liver are as marked as when the drug is given by the stomach.

It would seem that the acute transitory action of chloroform administered by the respiratory passages leads to a stimulation of hepatic metabolism, while the less acute and more sustained action leads to injury without previous stimulation.

Although in these experiments the administration of chloroform by the respiratory passages failed to cause the grave disturbances, metabolic and structural, which were caused by its administration by other channels, the results of such administration by other channels seem to show how, in the event of the chloroform given by the lungs not being eliminated with the usual rapidity, serious injuries might be effected, which would lead to the symptoms of late chloroform poisoning.

Moore and Roaf (*B.M.J.*, vol. ii., 1906, p. 721) have shown how chloroform is fixed to the proteins of the blood, and Nicloux (*C.R. Soc. biol.*, t. lx., pp. 206, 248, 1906; t. lxii., p. 1153, 1907) has also shown the way in which it is anchored to certain tissues. Sherrington and Sowton have proved that the action of chloroform upon the heart depends upon its tension in the circulating fluid, and that it is greater in saline solutions than in the protein-containing blood where it becomes fixed.

These observations, taken in conjunction with our results, seem to show that chloroform, when given by the respiratory passages, is rapidly taken up and first dissolved in the blood, and in this condition acts upon the nerve-centres, the excess being rapidly eliminated; while, when given by the stomach or hypodermically, the assumption is slow, more stable compounds are formed, the elimination is consequently delayed, and the drug has thus more time to produce a slow toxic effect upon the protoplasm of the tissues.

The onset of late chloroform poisoning after anæsthesia would thus be due to delayed elimination brought about by unusually firm fixation, or by respiratory deficiency.

(*Issued separately* August 15, 1908.)

On the Methods of Standardising Suprarenal Preparations.

By **I. D. Cameron**, M.B., D.P.H., Assistant to the Lecturer on Physiology, Edinburgh School of Medicine for Women. *From the Laboratory of the Royal College of Physicians, Edinburgh.*

(MS. received March 5, 1906.)

Since a number of preparations of the suprarenal capsules are now extensively used in therapeutics, it seems of some importance to determine the relative value of those most generally used. In the course of a series of experiments on the antagonism between nitrites and suprarenal preparations, and again between these organic extracts and those of the thyroid gland, it became necessary to determine the exact amount of the minimal effective dose. This afforded an opportunity of investigating the methods applicable to the standardisation of these preparations and their relative efficiency.

Since Takamine's * preparation of a pure product of adrenalin, many extracts of the suprarenals have been put on the market.

Meyer † has now established the precise nature of adrenalin and has prepared it synthetically, while Dakin ‡ has, also by synthesis, prepared a body closely allied to it and with a similar physiological action. It has now become possible for manufacturing chemists to prepare a practically pure and uniform product. To test how far uniformity exists in the present preparations, the following were examined :—

Adrenalin (Parke, Davis & Co.).
Suprarenalin (Armour & Co.).
Hemisine (Burroughs, Wellcome & Co.).

Several other commercial preparations were tested, but the results were found to be uncertain even with the administration of very large doses ; in a few cases a fall of blood pressure instead of a rise was given.

* *American Journal of Pharmacy*, 1901, p. 523.
† *Arch. f. Exper. Path.*, xxxv. p. 213.
‡ *Proc. of Roy. Soc.*, Oct. 7, 1905, p. 491.

Various methods of determining the strength of action of the suprarenal preparations have been proposed.

A.—*Chemical.*

Colorimetric methods were suggested by Battelli * and others. The reactions depend on the rose colour given with a weak solution of iodine and on the green tint produced with ferric chloride. The nature of these reactions is little understood, and the possibility of their being given by other substances is great. They do not necessarily afford a reliable test of the physiological activity of the product. I find that, while they afford a means of roughly standardising preparations of greater strength than adrenalin 1 in 40,000, it is impossible to use them in more dilute solutions. An additional difficulty is presented when the solution to be tested is already of a brownish colour. Hence they do not seem to afford a reliable or delicate method for standardising preparations.

B.—*Physiological.*

1. *Perfusion of Vessels of Frog.*—Läwen † perfused the frog's vessels and got a decided constriction with 0·002 mg. of suprarenin in 10 c.cm. (0·2 per million) under a pressure of 20 cm. of water.

2. *Action on Pupil of Frog's Eye.*—Ehrmann, as a preliminary to his investigation as to the fate of adrenalin in the blood, devised a method of standardising the solutions by their effect on the pupil of the frog's eye. To eliminate the sympathetic effect, he used enucleated bulbs. The controls were placed in physiological salt solution. The amount of dilatation of the pupil is expressed as "maximal," "almost maximal," "considerable" and "slight." He showed that 0·00005 mg. in 1 c.cm. (·05 per million) gave a slight dilatation of the pupil, while further dilutions gave no result.

On repeating these experiments of Ehrmann, similarly satisfactory results were not obtained. There is difficulty in measuring the exact amount of dilatation of the pupil. It is also possible that absorption through a fibrous membrane, especially under artificial conditions, may not be equal and constant. In one experiment, where the solution used was ·00025 mg. in ·5 cm. (0·5 per million),

* *Compt. Rend. Soc. de Biolog.*, 1902, p. 571.

† *Arch. f. Experim. Path. u. Pharmak.*, Bd. li., S. 415.

dilatation of the pupil resulted, but it was followed on long exposure by a marked contraction. Probably any slight injury to the eye during enucleation may interfere with the result.

3. *On Arterial Blood Pressure.*—Ehrmann, who published his results when my investigations were nearly completed, also tested the minimal effect on the blood pressure in cats. He quotes one experiment in which 0·08 mg. (0·02 mg. in 1 c.cm. of which 4 c.cm. were injected) gave a slight increase of blood pressure. The effect was just appreciable when five-eighths of that quantity was used. In these experiments the anæsthetic was ether, and the injections were made into the jugular vein.

Elliott,* in investigating the disappearance of adrenalin from the body, estimated its strength by means of the blood pressure in cats under urethane and with cut vagi. The maximal pressure curves given with increasing doses of adrenalin injected in equal times were found. The intervening values were estimated from these curves. The rise varied with the vein into which the injection was made, being greatest in the external jugular. The blood pressure results were corroborated by the typical eye reactions—widening of the eyelids, dilatation of the pupil, and retraction of the nictitating membrane.

Before the publication of Ehrmann's and Läwen's papers, I had investigated the availability of the perfusion method and of the method of determining the effect on arterial pressure.

In the perfusion experiments, a cannula was tied into the aorta of a pithed frog, and the fluid to be tested was allowed to flow by gravitation from a reservoir. After passing through the vessels of the frog, the fluid escaped through the cut sinus venosus. As the difference in viscosity of the perfused fluids was practically negligible, the effect of each could be determined by the rate of flow from the cut sinus. To avoid the laborious proceeding of counting the drops, a graphic record was taken. The perfused fluid, as it escaped, was allowed to drop on a light aluminium plate, $\frac{3}{4}$ inch square, which was fixed obliquely to the free end of a lever. The movement of the lever caused by the fall of a drop of fluid was transmitted by means of tambours to a second lever, to the

* *Journal of Physiology*, vol. xxxii. p. 447.

end of which a writing style was attached. A record was made on a revolving drum. By this means, and by an electric marker which marked half minutes, the rate of flow was graphically represented.

The solutions were, in all cases, made with Locke's modification of Ringer's solution. It was found necessary to make the solution immediately before use, as the fluid became pink, and rapidly lost its activity.

1. *Perfusion of the Vessels in the Pithed Frog.*—Perfusion of 1 per million gives a very marked constriction, which continues for a considerable time after the adrenalin is withdrawn and Locke's solution is again perfused. Recovery, however, takes place (unless the constricting fluid has been perfused for too long a time), and the normal rate is re-established. Further dilution to 0·5 per million gives a considerable constriction, while there is still a feeble reaction with a solution of ·1 in one million.

The following experiment shows the slight constricting action of 0·1 per million :—

After perfusion for some time.		Solution.
Time.	Drops per minute.	
3·50	24	Locke's solution.
3·53	24	
3·56	24	
3·58		Adrenalin 0·01 per cent. of 1 in 1000, 0·1 in a million.
4·1	20	
4·2	21	
4·3	20	
4·4	20	
4·5	20	
4·8	20	Locke's solution.
4·11	20	
4·14	22	
4·16	23	
4·17	22	
4·18	24	

When this result is compared with Läwen's, a great similarity is seen.

He got marked constriction with ·002 mg. in 10 c.c. suprarenin, *i.e.* with 0·2 per million.

The results on different frogs were somewhat variable, and further, the method of perfusion is tedious and troublesome, especially if the preparation to be tested is used after the action of a preparation of standard strength.

2. *Minimal Effective Dose on Blood Pressure in Rabbits.*—The blood pressure experiments were performed by Dr Noël Paton. Rabbits of fairly uniform size (about 2000 grams) were selected. The carotid artery was connected with a mercury manometer in the usual way, and the injections were made into the jugular vein. Full anæsthesia by ether was maintained till the death of the animal.

Gradually decreasing doses were injected into the jugular vein. The smallest amount which gave a definite and invariable rise of blood pressure was 0·5 c.c. of a 0·125 per cent. solution of adrenalin (Parke, Davis, 1 in 1000)—that is, ·00062 mg. of adrenalin, or ·0003 mgs. per kilo. A similar result was given with hemisine and with suprarenalin when used in the same strengths (fig. 1). In the case of the adrenalin and of the suprarenalin, the solution was made from the commercial 1 in 1000 preparation : the hemisine tabloid was first made up to 1 in 1000 with Locke's solution, and was then further diluted as required. This result may be expressed in another way—·00000031 gram per kilogram of body weight causes the minimal rise of blood pressure in rabbits. If the blood of the rabbit is taken as 5 per cent. of its body weight,* then ·006 per million of blood is effective. When we compare this result with that of Ehrmann,† who found that ·00002 gram per kilogram of body weight in a cat gave a rise of pressure which was just appreciable, we find a corroboration of Lesage's ‡ statement as to the comparative resistance of the cat to the effect of adrenalin. In an experiment on a cat of two and a half kilogrammes, a rise of blood pressure was given with ·00003 g. in 1 c.c. (·000012 g. per kilo)—that is, just over one-half the amount given by Ehrmann to produce his minimal result. It is interesting to compare this result with Schäfer's § suggestion, made before

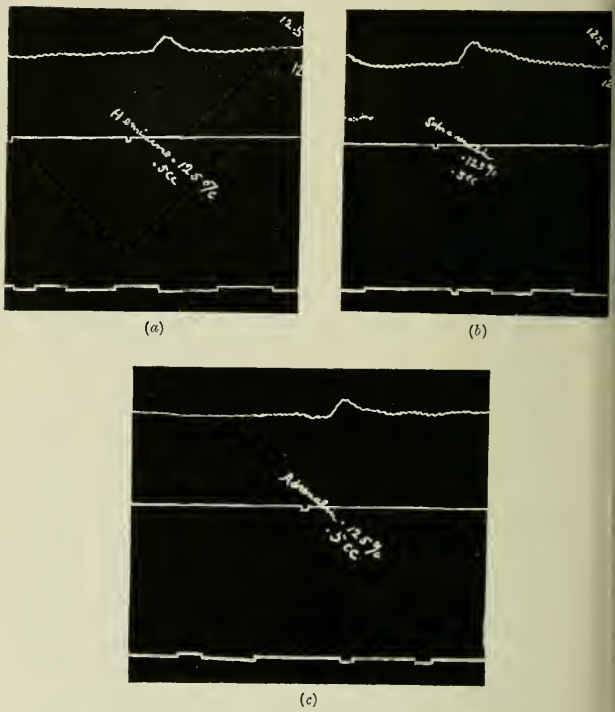
* Douglas, *Journal of Physiology*, vol. xxxiii. p. 493.

† *Loc. cit.*

‡ *Arch. intern. de Pharmac. et de Thérap.*, Ii. p. 304, and *C. R. Soc. Biol.*, 1904, p. 665.

§ *Text-book of Physiology*, vol. i. p. 957.

the active principle was isolated, that ·000001 g. per kilogram of body weight would produce a distinct action of the heart and arteries.



(*a*) (*b*)

(*c*)

FIG. 1. To show the effect on the blood pressure of intravenous injection in the rabbit of ·00031 mg. per kilo of (*a*) Hemisine; (*b*) Suprarenalin; and (*c*) Adrenalin.

The same rise of pressure was given whether the initial pressure was low or high.

* In all traces time is marked in half minutes.

A similar curve of increased pressure was given when the same amount of adrenalin was injected towards the end of a fall of pressure from sodium nitrite (fig. 2), and during the slowly rising pressure produced by a weak solution of barium chloride (·5 c.c. of ·5 per cent. solution).

This weak solution, then, ·0000031 gram per kilo of body weight, produces a distinct effect when the blood pressure is normal, when it is falling gradually, and when it is being increased slowly.

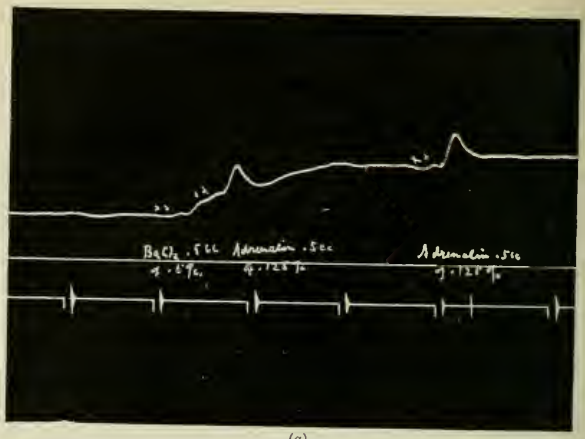
With a reliable adrenalin preparation available to give a standard of comparison, this method affords an excellent and easy means of standardising any preparation. But when this is not available the method is not so satisfactory.

3. *Antagonism to definite Doses of Vaso-dilators.*—On account of the disadvantages of these methods, the plan of standardising against a vaso-dilator of known composition was tried. The marked peripheral vascular dilatation caused by the nitrites suggested their use. It is recognised that all the nitrites cause vascular dilatation, but their effects differ in degree and in duration. The nitrate esters which act as nitrites were not available because of their insolubility. As Atkinson * points out, nitrous acid is too unstable to be used. The amyl base in amyl nitrite has a decided action of its own, and the alcohol required to keep it in solution increases the dilatation. Sodium has not a well-marked pharmacological activity, and so sodium nitrite is a convenient salt to use in order to get a nitrite effect.

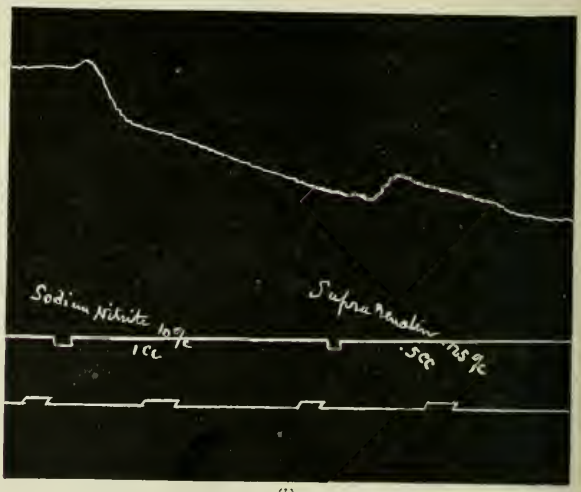
Hay † states that nitroglycerin is chemically a nitrate of glyceryl; in an alkaline fluid such as the blood, it is decomposed and a nitrite is liberated. Marshall ‡ does not agree with this view of the action of nitroglycerin. He contends that it probably remains unchanged and is not reduced until it reaches the tissue cells. Wherever the seat of the chemical change may be, there is no doubt that nitroglycerin acts physiologically as a nitrite.

Sodium nitrite was first used in this series of experiments.

* *Journal of Anatomy and Physiology*, vol. xxi. p. 225.
† *Practitioner*, vol. xxx., 1883, p. 422.
‡ *Journal of Physiology*, 1897–98, p. 1.



(*a*)



(*b*)

FIG. 2. To show the effect of a minimal dose of adrenalin on the blood pressure of a rabbit during (*a*) a rise from injection of barium chloride, and (*b*) towards the end of a nitrite fall of pressure.

Many commercial preparations of sodium nitrite are very impure and contain considerable quantities of the nitrate. The sodium nitrite of Merck is practically pure. The crystals were kept dry and the solutions were freshly made. When made in this way, the solution of nitrite is practically of uniform strength.

Nitrites have been largely used in perfusion experiments. Atkinson * perfused frogs with solutions of sodium nitrite (containing 99 per cent. of nitrite by the permanganate method), and he got dilatation of the vessels in solutions of from 1 in 100 to 1 in 100,000—the increased flow in the latter case being from 16–18 per cent. A solution of 1 in 200,000 was without effect. He also perfused nitroglycerin, and got an increased flow up to a dilution of 1 in 1,000,000.

Leech † perfused decapitated tortoises with solutions of sodium nitrite and collected the fluid in graduated flasks. After a preliminary contraction, dilatation of the vessel wall resulted with strengths of from 1 in 1000 to 1 in 10,000. This preliminary contraction was not seen on perfusing the kidneys of warm-blooded animals. He also found marked dilatation on perfusion of the lungs of a cat killed by chloroform. Leech calls attention to a certain resemblance between the action of alcohol and that of the nitrites, but he states that the nitrites are more rapid and also more evanescent in their action. Marshall,‡ in the course of his experiments on the antagonism of digitalis and the nitrites, perfused sheep's kidneys with sheep's blood, and demonstrated that there was not chemical antagonism, but that each drug produced its own effect, which varied with the length of time and the rapidity of action, and also with the strength of the solution.

Both the perfusion method in pithed frogs and blood pressure in rabbits were used as a means of estimating the effect of adrenalin and a nitrite given together.

(*a*) *Perfusion.*—A solution of adrenalin of the strength of 0·05 per cent. of 1 in 1000 gives a marked constriction when perfused in a pithed frog. Sodium nitrite (Merck) was added

* *Loc. cit.*

† *Brit. Med. Journal*, vol. i., 1893, p. 1305. ‡ *Loc. cit.*

to the solution. Until 6 per cent. of the nitrite was added, the adrenalin constriction was still given. When this strength was reached, no constriction appeared even with continued perfusion, but a slight dilatation was present. When the perfusion of the mixture was stopped and Locke's solution was used, the nitrite effect rapidly passed off, and a prolonged adrenalin effect was given. The nitrite apparently could only antagonise the effect of the adrenalin or delay its action while the two drugs were being perfused through the vessels.

A typical experiment in which antagonism was not established may be given, as it demonstrates the influence of both drugs on the vessels.

After perfusion for 35 minutes.		Solution.
Time.	Drops per minute.	
1·25	20	Locke's.
1·27	20	
1·28		Sod. nitrite, 0·5 per cent.
1·38	46	
1·41	44	
1·43	48	
1·48	44	
1·50		Locke's.
1·56	40	
2·0	40	
2·4	28	
2·8	22	
2·25		Sod. nitrite, 0·25 per cent. ;
2·28		adrenalin, 0·05 per cent.
2·35	6	
2·38	10	

(*b*) *Blood Pressure.* — The effect of nitrites on the blood pressure has been extensively studied.

Lauder Brunton * investigated the fall of blood pressure caused by the inhalation of amyl nitrite. Atkinson,† in experiments on rabbits with their vagi intact, got a fall of blood pressure and acceleration of the pulse with a dose of $\frac{1}{2}$ grain of sodium nitrite. A similar result was obtained with much smaller doses of nitroglycerin. The pressure fell with 1 in 1500, but with 1 in 50,000

* *Journal of Anatomy and Physiology*, vol. v. p. 92.

† *Loc. cit.*

there was a slight rise. In blood pressure experiments with a mixture of digitalin and nitroglycerin, Marshall * found that, as in his perfusion experiments, each drug gave its own characteristic action, the nitrite fall of pressure, being a less prolonged effect, passed off, and then a digitalin rise was seen. This result was corroborated on the isolated frog's heart.

Leech † has tested the action of barium nitrite on muscle directly. Barium chloride increases the contractile power of muscle and causes contracture. In barium nitrite, the two parts appear to act independently. The barium action is first evident, later the contraction is annulled, and death of the muscle results earlier than with barium chloride.

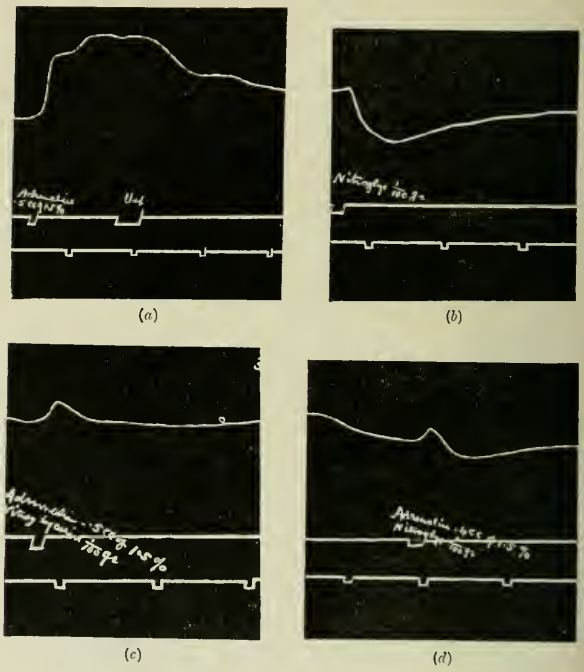
In the present experiments rabbits were used, and the blood pressure was estimated as described above. Nitroglycerin was here used in order to get a more rapid effect than that given with the sodium nitrite. The preparation used was that of Parke, Davis & Co., made up in $\frac{1}{100}$ grain (0·6 mg.) tablets.

Adrenalin was injected, ·5 c.c. of 1·5 per cent. (fig. 3 *a*), and when the pressure had again returned to normal, the nitrite fall was demonstrated with nitroglycerin $\frac{1}{100}$ grain (0·6 mg.) (fig. 3 *b*). Then an equal volume of solution which contained both drugs was injected.

When adrenalin 0·5 c.c. of 1·5 per cent. was injected with 1 c.c. containing 0·6 mg. grain of nitroglycerin, the adrenalin rise was almost entirely cut down, and the opposing effect of the nitroglycerin was soon manifested by a very slight fall and then a return of the normal pressure (fig. 3 *c*). The tracing given closely resembles a minimal adrenalin effect. Each of the drugs is here apparently acting independently, but in opposite directions—the greater rapidity of action of the adrenalin showing itself in the increase of blood pressure before the nitrite acts. If the amount of adrenalin injected be still further diminished (·4 c.c. of 1·5 per cent. being injected) while the same amount of nitroglycerin is used, the effect of the change in proportion is seen (fig. 3 *d*). Again, the rapid action of adrenalin causes an increase of pressure which is soon abolished by the commencing nitrite action. In this case a sufficient amount of nitrite is given, not only to

* *Loc. cit.* † *Loc. cit.*

prevent the adrenalin rise, but also to cause a definite fall of pressure. This fall is not, however, very marked, as the vascular dilatation is being opposed although not abolished by the adrenalin.



(*a*) (*b*) (*c*) (*d*)

FIG. 3. To show the neutralising action on the blood pressure of nitroglycerin and adrenalin. (*a*) Effect of adrenalin 0·0075 mg.; (*b*) effect of nitroglycerin 0·6 mg.; (*c*) effect of (*a*) and (*b*) combined; (*d*) effect of adrenalin 0·006 mg. with 0·6 mg. of nitroglycerin.

The best antagonistic action is thus given with ·5 c.c. of 1·5 per cent. solution of adrenalin combined with $\frac{1}{100}$ grain of nitroglycerin—·0075 mg. of adrenalin is antagonised by ·6 mg. of nitroglycerin—that is, the nitroglycerin and adrenalin are as 80 to 1.

In an experiment on a cat, the amount of adrenalin which produced a moderate rise of pressure (·03 mg. in 1 c.c.) was combined with $\frac{1}{100}$ grain of nitroglycerin, and a result very similar to that given with the smaller dose in rabbits was given (fig. 4).

The preliminary rise of adrenalin is again inhibited by the action of the nitroglycerin, and the normal pressure level is rapidly reached. It is interesting to note that although the adrenalin must be used in strong solutions in order to effect the blood pressure in the cat, nitroglycerin apparently acts in very much the same way in the cat as it does in the rabbit. This also tends to prove that adrenalin

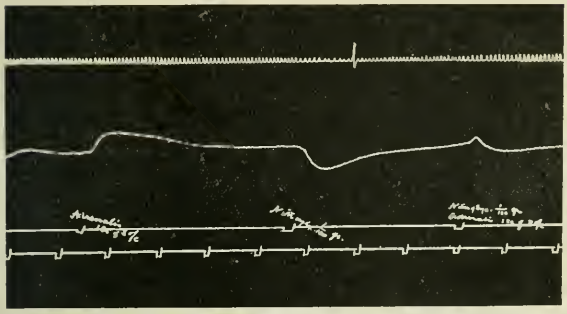


Fig. 4. To show the neutralising effect of nitroglycerin and adrenalin on the blood pressure of the cat, ·03 mg. of adrenalin against 0·6 mg. of nitroglycerin.*

and a nitrite act independently but in opposite directions—in other words, that the antagonism is what might be called dynamic rather than chemical.

Of these two methods employed for the combination of nitrite and adrenalin, that of estimation by the blood pressure is more satisfactory. The action of adrenalin when perfused through the peripheral circulation is of considerable duration, while a return to the normal is more rapid with a nitrite. On long-continued perfusion then, although at first an antagonism is established, the adrenalin effect ultimately appears on discontinuing the perfusion. The power of the cells to react to the stimulus of nitrite is more readily exhausted; then the adrenalin effect is seen.

* Top line is the respirations.

In my hands the method of standardising preparations of adrenalin which has proved most serviceable is that of testing their antagonistic effect to a known dose of nitroglycerin, 0·6 mg. ($\frac{1}{100}$ gr.) of which should require 0·0075 mg. of the adrenalin preparation to antagonise its dilator action.

In one preparation of suprarenal submitted to the Laboratory for examination, the minimal effective dose was 0·5 c.c. of a 1 per cent. solution. The minimal effective dose of adrenalin is 0·00062 mg. for a rabbit of about 2000 grams. Of the solution tested, ·005 c.c. is then equal to ·00062 mg. of adrenalin—that is, 1 c.c. = 0·12 mg.

This result is corroborated when the nitroglycerin method of standardisation is used. The dilator effect of $\frac{1}{100}$ grain nitroglycerin (·6 mg.) is opposed by ·7 c.c. of 10 per cent. The same amount of nitroglycerin is neutralised in effect by ·5 c.c. of 1·5 per cent. of adrenalin (·0075 mg.). Then 1 c.c. of the solution is equal to 0·107 mg.

The solution is therefore rather more than $\frac{1}{100}$ as strong as the adrenalin chloride 1 in 1000 of Parke, Davis & Co.

Summary.

For the standardisation of adrenalin preparations.

(*a*) The colorimetric method is not reliable with weak or impure solutions.

(*b*) The effect on the pupil of the frog's eye gives uncertain results.

(*c*) The determination of the minimum effective dose on the arterioles of the perfused frog is tedious and uncertain; on an average 0·1 per million produces an effect.

(*d*) The determination of the minimum effective dose in causing a rise on the blood pressure of the atropinised rabbit yields fairly satisfactory results.

(*e*) Adrenalin, suprarenalin, and hemisine all give a precisely similar result, 0·0003 mg. per kilo of body weight, or 0·006 per million, of the rabbit's blood causing a distinct rise in the blood pressure in the rabbit, and 0·012 mg. per kilo of body weight, or 0·24 per million, of the blood causing a rise in the cat.

(*f*) The most satisfactory method is the determination of the dose just sufficient to antagonise 0·6 mg. ($\frac{1}{100}$ gr.) of nitroglycerin (Parke, Davis & Co.). Of adrenalin, 0·0075 mg. is sufficient.

I wish to express my indebtedness to Dr Noël Paton for much help in the preparation of this paper.

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The Action of Certain Salts of Formic Acid on the Circulatory and Muscular Systems.

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I. Introductory.

The formates at the present time are receiving much notice. Points of interest are associated with their chemistry, and in

the realm of therapeutics no up-to-date tonic is complete without them. It has been claimed for them that they stimulate the heart, vessels, bladder and alimentary canal; that they increase the appetite, abolish fatigue, and exert a tonic influence on muscles. One enthusiast suggests that for the latter reason formic acid should replace alcohol among the working-classes. It has been stated that the formates are powerful diuretics; that they conserve youth, delay senescence, and that they cure rheumatism, tremors and pneumonia. When administered to rabbits they increase their vivacity, while the endowment of the ant with formic acid explains its industry.

The local application of formic acid is said to cure lupus and inoperable cancer, and it has been recommended as a preservative of food. The physiological action of substances alleged to be possessed of so many of the attributes of an elixir of life seemed to us to be worth investigating.

The literature on the subject is for the most part unsatisfactory. The work of Clément[1] and Garrigue[2] can only be described as that of wild enthusiasts. Huchard,[3] however, to a certain extent confirms their results, though he does so in more restrained phraseology.

A much more careful piece of work is that of Fleig.[4] In the first part of his paper he criticises his French confrères so unmercifully and so thoroughly that he leaves subsequent critics very little to say. Indeed, had Fleig's paper received the attention it deserves the formates would never have attained the position in fashionable therapeutics they now have. Fleig immersed exposed loops of intestine in animals in Locke's fluid and noted the movements. Formate of soda added in varying proportions never affected the contractions in amplitude, frequency, or rhythm. Experimenting with the isolated heart of rabbits he never obtained an excitor or reinforcing effect due to sodium formate. On the contrary, concentrated solutions caused toxic effects, lessened amplitude, diminished frequency and arhythmia.

In the case of a nearly exhausted heart, however, calcium formate acted as a stimulant, just as any calcium salt would. The contractions of the gastrocnemius of rabbits set up by induction shocks were not affected by sodium formate.

Nerve energy direct and reflex was not stimulated by sodium formate. Ergographic records, carefully carried out, were negative

in Fleig's observations, and he ascribes the results of Clément and Huchard to the effects of training and suggestion.

Among the purely clinical records the only observations of importance are those of Croom[5] in 1906, and Ker and Croom,[6] published last June. They have given formic acid to patients suffering from diphtheria, and conclude that the results they obtained are distinctly encouraging, particularly in relation to the occurrence of cardiac failure and paralysis. They, however, themselves admit that standards of comparison for its merits are difficult to obtain in such a disease. They cannot confirm the diuretic effect observed by Clément and Huchard, and in this particular standards of comparison are definite. While their general verdict on formic acid is favourable, it is expressed with an amount of Scotch caution which contrasts strongly with the enthusiasm of the French advocates.

The salts we employed in the following experiments were those of sodium, potassium and calcium. In many instances, however, the action of the two latter was evidently affected, sometimes to a preponderating extent, by the metallic ion, so that the bulk of our later investigations were carried out with the sodium salt.

A few observations were made with formic acid, but its powerful local noxious effects in causing tissue necrosis and increasing coagulability of the blood obviously rendered it unsuitable for such investigations as we wished to carry out.

We may note in passing that the formic acid, even in dilute solution, has a local effect on muscle. The muscle becomes more translucent and acquires a curious glazed appearance.

II. Observations on Animals.

1. *Effects on Blood Pressure.*—Observations were made on 14 rabbits, fully anæsthetised with ether. In most cases several salts were employed. Tracings were taken from the left carotid, and the solutions were injected into the left jugular vein. The vagus nerves were cut in the great majority of cases. The doses varied from 10 to 20 m. according to the weight of the rabbits.

Sodium Formate.—

Injection of a 1/500 solution had no effect.

Injection of 1/100 solution in 4 instances, and of 1/50 solution in 2 instances, had no effect.

Injection of 1/25 caused a very slight fall of pressure in 3 cases; but in one instance had no effect.

Injection of 1/10 caused a slight fall, with immediate recovery in 5 cases.

Saturated solution caused a fall of 64 mm. of mercury, followed by a gradual rise to normal.

Potassium Formate.—

Injection of 1/1000 caused a slight fall of pressure, with immediate recovery.

Injection of 1/100 caused a fall of pressure, with recovery in 3 cases, and was negative in 3 cases.

Injection of 1/25 in all of 3 cases caused a very definite fall, with recovery gradual and not always complete.

Injection of 1/10 in all of 4 cases caused a fall practically to zero, and was immediately fatal in 3 cases. The fourth animal recovered only after artificial respiration.

Calcium Formate.—

Injection of 1/500 had no effect.

Injection of 1/250 had no effect.

Injection of 1/100 caused a slight fall in one case, a slight rise in another, had no effect in a third, and stopped the experiment by clotting in two instances.

Two injections of 1/50 had no effect.

Injection of 1/25 caused a slight fall, with recovery in 2 cases, and had no effect on a third.

Two injections of 1/10 (saturated) solution tended to cause a slight rise. In one case a slight fall was caused. In each case clotting occurred and prevented further observations.

Formic Acid.—Two injections of 1/50 were made. In each case, after a preliminary fall and recovery, there was a long and gradual fall.

These experiments indicate that sodium formate has little effect on the blood pressure, unless it is in fair concentration. Strong solutions are definitely depressor.

Potassium formate is slightly depressor in dilute solutions, definitely depressor in moderate concentration, and usually fatally inhibits the heart in strong solutions.

The experiments with calcium were less definite, but they

probably warrant the inference that the more dilute solutions have a slight depressor effect, and that concentrated solutions tend to raise the pressure.

The general effect of the acid in the two experiments was depressor.

The want of similarity in the action of the different salts is to be explained by the influence of the metallic ion. The true formate effect is shown in the case of the soda salt to be depressor.

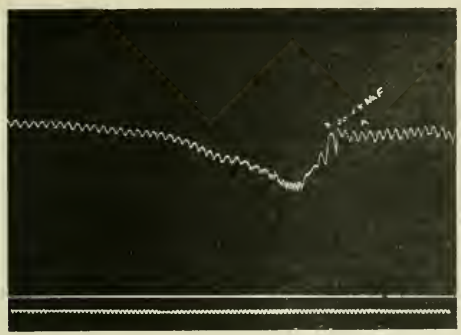

FIG. 1.—Blood pressure tracing from left carotid of rabbit, showing fall of pressure following the intravenous injection (between the two crosses) of 20 m. of sodium formate (1 in 5). Trace reads from right to left.

This depressor effect becomes enormous when added to the preponderating potash effect, while it tends to be obscured altogether when opposed by the stimulating action of calcium.

2. *Observations on the Blood-Vessels.*—These experiments were carried out on pithed frogs. A cannula was tied into the aorta and Ringer's solution was allowed to flow into it from a reservoir in which the fluid was kept at approximately the same level. The salt to be investigated was added to the Ringer's solution in known proportions. After flowing through the vessels the fluid escaped from a cut in the sinus venosus, and eventually dropped from the animal's toes. By such an arrangement any change in the calibre of the vessels affects the rate at which the drops fall. We used a

graphic method for recording the drops. They were allowed to fall on a piece of tin-foil attached to the long lever of a receiving tambour. The movements were transmitted to the lever of a recording tambour and were marked on the smoked paper of a kymographion. Time intervals were recorded by means of an electric marker. After a salt had been perfused the vessels were again perfused with Ringer's solution only. Those experiments, in which the return rate most closely approached the original rate, were obviously the most satisfactory, but a failure to obtain a return rate similar to the original does not necessarily invalidate the observation. Sixty-six experiments were carried out. The following is a typical example:—

Frog perfused with Ringer's Solution.

Original Rate . . .	20 per minute.

After the addition of 1 *in* 1000 *Sodium Formate.*

In 30 seconds . . .	22 per minute.
In 60 seconds . . .	24 „
1 to 3½ minutes . . .	28 „
3½ to 5½ minutes . .	26 „
5½ to 6 minutes . . .	24 „
6 to 7 minutes . . .	22 „
7 to 10 minutes . . .	20 „
10 to 14 minutes . .	18 „

Ringer's Solution only.

14 to 19 minutes . .	16 per minute.
19 to 20 minutes . .	18 „

With a few exceptions it was found that all the salts in all the dilutions used first caused a dilatation followed by constriction.

In 15 instances (7 potassium, 4 soda, 4 calcium) there was no dilatation, and in 10 instances (8 soda, 1 potassium, 1 calcium) no constriction occurred. In nearly every case the dilatation began within 3 seconds, never later than 30 seconds, and gradually approached its maximum. The constriction followed in from 17 to 480 seconds, usually about the expiry of a minute and a half. Although the intervals of time at which the dilatation and constriction occurred varied within very narrow limits, it seemed to us that in collating our results to select arbitrary intervals and state

the conditions then found would not correctly indicate the result of our experiments. We therefore chose the maximum amount of change in each case.

The results are tabulated as follows.

The number of drops is that counted in 30 seconds.

Sodium Formate, 1 *in* 1000.

Experiment.	Original Rate.	Maximum Dilatation Rate.	Maximum Constriction Rate.	Return Rate.	Percentage Rate.	
					Dilatation.	Constriction.
1	108	127	99	99	117	91
2	20	22	14	19	110	70
3	19	19	14	19	100	73
4	33	47	16	...	142	52
5	136	151	114	130	111	83
6	12	16	10	10	133	83
7	142	...	115	171	100	81
8	38	56	56	56	145	100
9	34	37	12	12	109	35
			Average . .		118	74

Sodium Formate, 1 *in* 500.

10	99	104	77	77	110	80
11	19	23	19	19	121	100
12	19	23	19	19	121	100
13	8	9	5	6	112	62
14	68	130	...	120	191	100
15	10	...	8	...	100	80
			Average . .		126	87

Sodium Formate, 1 *in* 250.

16	77	96	71	71	124	78
17	19	26	19	19	136	100
18	19	25	16	16	131	84
19	7	10	7	7	142	100
			Average . .		133	90

Sodium Formate, 1 *in* 100.

Experiment.	Original Rate.	Maximum Dilatation Rate.	Maximum Constriction Rate.	Return Rate.	Percentage Rate.	
					Dilatation.	Constriction.
20	20	...	2	10	100	90
21	71	75	60	60	105	84
22	19	25	18	18	132	94
23	16	21	5	11	131	69
			Average . .		117	84

Sodium Formate, 1 *in* 50.

24	60	180	60	60	300	100
25	18	24	10	18	133	55
26	11	19	9	9	181	81
			Average . .		204	78

Sodium Formate, 1 *in* 25.

27	60	166	60	60	276	100
28	18	29	4	...	161	78
29	9	11	4	...	122	56
			Average . .		186	78

Sodium Formate, 1 *in* 10.

30	5	7	...	6	140	100
31	19	39	12	15	200	63
			Average . .		170	81

Potassium Formate, 1 *in* 1000.

32	188	...	125	136	100	66
33	10	11	7	10	110	70
34	171	...	129	156	100	75
35	56	...	48	56	100	85
36	45	47	39	42	104	86
37	60	67	36	55	112	86
38	33	46	17	26	139	51
39	9	12	10	9	133	100
			Average . .		112	77

Potassium Formate, 1 *in* 500.

Experiment.	Original Rate.	Maximum Dilatation Rate.	Maximum Constriction Rate.	Return Rate.	Percentage Rate.	
					Dilatation.	Constriction.
40	6	11	5	7	183	63
41	120	148	36	120	123	30
42	42	44	18	38	104	41
43	44	59	36	55	134	81
44	50	59	47	50	118	94
			Average . .		132	62

Potassium Formate, 1 *in* 250.

45	8	8	3	8	100	37
46	30	34	7	26	113	20
47	55	...	25	40	100	45
48	50	57	39	51	114	78
			Average . .		107	45

Potassium Formate, 1 *in* 100.

49	26	34	2	20	130	17
50	40	...	21	...	100	52
			Average . .		115	34

Potassium Formate, 1 *in* 50.

51	20	...	3	...	100	15

Potassium Formate, 1 *in* 10.

52	25	29	13	19	115	52

Calcium Formate, 1 *in* 1000.

53	130	...	10	...	100	8
54	10	...	6	8	100	60
55	156	158	65	68	101	41
56	56	...	4	58	100	7
57	51	93	41	52	183	81
58	50	58	37	46	116	74
			Average . .		116	45

Calcium Formate, 1 *in* 500.

Experiment.	Original Rate.	Maximum Dilatation Rate.	Maximum Constriction Rate.	Return Rate.	Percentage Rate.	
					Dilatation.	Constriction.
59	50	60	23	49	120	46
60	19	30	12	15	160	78
61	27	36	24	27	133	88
62	7	10	5	7	142	71
			Average . .		139	71

Calcium Formate, 1 *in* 250.

63	15	20	...	15	133	100
64	8	8	3	...	100	37
65	15	19	11	7	126	57
			Average . .		120	64

Calcium Formate, 1 *in* 10.

66	7	10	5	...	142	71

These tables show (1) that the sodium salt caused the greatest individual amount of dilatation, that the average amount of dilatation is greater than in the case of the other salts, and that sodium formate has the least constrictor effect.

(2) The potassium salt caused the greatest individual amount of constriction, the greatest average amount of constriction, and the least individual and least average amount of dilatation.

(3) The calcium salt had effects on the vessels intermediate between those of sodium and potassium.

(4) As regards strength of solution it is seen that in the case of soda and calcium the amount of dilatation increases in proportion to increasing strength of solution, and that the amount of constriction diminishes with increasing concentration.

In the case of potassium the amount of dilatation remains constant for all the concentrations used, while constriction increases with increased strength of solution.

3. *Observations on the Heart.*—(A) *Perfusion Experiments.*—Frogs' hearts were used. The perfusions were carried out by

means of Schafer's heart plethysmograph. In some of the cases instead of using the piston recorder of Schafer's instrument we attached the very delicate and simple volume recorder recently introduced by Dixon.[7]

Sodium Formate.—Seven observations were made with 1 in 1000 solution.

In two cases results were negative.

In three instances the heart was slowed and then stopped in diastole.

In two the heart became irregular and was stopped in diastole. In the latter five cases the force of contraction was greatly diminished.

Sodium formate 1 in 100 either perfused through the heart or merely painted on it, rapidly decreased the force of contraction, and in four out of five cases stopped the heart in diastole.

Sodium formate 1 in 10 caused great irregularity, with frequently an increased rate and speedy stoppage in diastole.

Potassium formate 1 in 1000, 1 in 500, and 1 in 100 caused irregularity, diminished force, and stopped the heart in systole.

Potassium formate 1 in 10 immediately stopped the heart.

Calcium formate 1 in 1000 augmented the force of the heart, sometimes caused irregularity, and soon stopped it in systole.

Solutions of 1 in 500, 1 in 100, and 1 in 10, slowed and stopped the heart in systole.

Formic acid 1 in 2000 diminished the force of contraction, and stopped it in systole.

Hearts perfused with formic acid showed a remarkable increase in translucency, in some cases becoming glassy-like and almost transparent.

Some points of interest emerged in the inter-relationship of the action of these salts. Thus a heart stopped in diastole by sodium formate could sometimes be made to beat again by perfusing calcium formate, but only for so long as the calcium formate was perfused.

A heart brought to a standstill by dilute calcium formate could be made to beat again for a brief period by perfusing strong sodium formate through it.

(B) *Heart Movements.*—These observations were also made on frogs' hearts. After the frog was pithed a hook was passed through the tip of the ventricle and attached by a pin to an aluminium

lever writing against a smoked drum. The salts were applied to the heart by dropping the solutions from a glass pipette. Fifteen such experiments were carried out, the results were confirmatory of the foregoing. Thus, solutions of potassium and sodium formate caused arhythmia, followed in succession by diminished rate, diminished force with increased rate, diminished force with lessened rate, and speedy stoppage with strong solutions. The

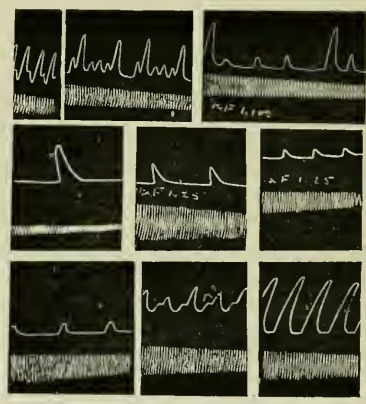

Fig. 2.—Frog's heart trace. 1. Shows effect of painting potassium formate (1 in 100) on the heart 2. Shows effect of sodium formate on a heart nearly stopped by potassium formate. 3. Shows effect of painting calcium formate on the same heart.

potassium salt acted more powerfully than the sodium. Calcium formate 1 in 100 produced slight slowing of the heart only. Solutions 1 in 10 not only slowed the heart but lessened its force of contraction.

The application of calcium formate in several instances caused a return of contraction in hearts stopped by the application of the potassium or sodium salts. The two series of observations indicate that all three salts are toxic to the heart. In the case of potassium formate the toxic action is practically exclusive. In

the case of sodium formate a certain stimulating effect, probably in virtue of irritation can also be noted. The effect of calcium formate is mainly that of calcium, the toxic effects being secondary.

4. *Observations on Muscle Curves and Fatigue.*—The gastrocnemius of the frog was used. The muscle of one limb was made to record the simple contraction induced by an induction shock of optimum strength, and was then either injected or painted with a

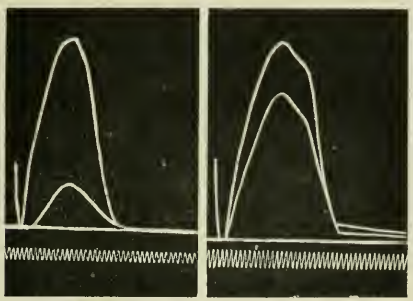

Fig. 3.—Left figure shows gastrocnemius contraction before and after injection of sodium formate (1 in 100) 3 m. into the muscle. Right figure shows the contraction of the opposite gastrocnemius before and after injection of 3 m. of Ringer's solution. The second curve is the smaller in each case.

formate. The corresponding muscle of the other limb was treated similarly with Ringer's solution. The same apparatus was used for the two limbs, the order of making the contrasted observations being alternated in successive experiments. In every case the muscle was loaded with 30 grammes.

It may be mentioned here that in no case did the Ringer's solution materially alter the height of the curve or the length of the latent period. Only the fatigue effects therefore need be stated.

Sodium formate 1 in 1000. Muscle injected with 5 m.

Curves before and after were the same height. Completely fatigued after 600 shocks. Ringer preparation fatigued but still active.

1 in 100. Muscle injected with 5 m. Curve lowered 1 mm. Completely fatigued after 450 shocks. Ringer preparation showed only lengthening of latent period.

1 in 10, 5 m. Curve lowered 6 mm. Latent period greatly increased. Fatigued after 200 shocks.

Saturated, 5 m. Curve lowered 8 mm. Latent period increased. Fatigued after 150 shocks. Ringer muscle not affected.

Potassium Formate.—

1/50. Curve lowered 2·5 mm.—fatigued after 400 shocks. Ringer preparation—fatigued after 800 shocks.

1/25. Curve lowered 3·5 mm.—fatigued after 250 shocks. Ringer muscle—fatigued after 600 shocks.

1/10. Curve lowered 5·5 mm.—fatigued after 100 shocks. Ringer muscle—fatigued after 600 shocks.

Saturated.—No response after injection.

Calcium Formate.—The height of the curves was somewhat diminished, but a great prolongation of contraction period became evident, due to calcium effect.

Formic Acid.—1/100 painted on the muscle lowered height of curve by 1 mm. Fatigue occurred after 200 shocks.

Ringer muscle—fatigued after 600 shocks.

These experiments indicate that the effects on skeletal muscle are similar to those on cardiac muscle—namely, that all three are toxic in the order, potassium, sodium, calcium, but that in the case of the latter the toxic effect is subsidiary to the action of the metallic radicle.

III. Observations on the Human Subject.

Although we now felt convinced that the administration of formates might be harmful, we made some observations upon ourselves, upon a lady student and a laboratory boy. Conditions as regards work and exercise were practically constant during the experiments. Three of the subjects were habitual abstainers from alcohol and tobacco: the fourth acquired these virtues for a month previous to the experiments, and during their course. Only sodium

formate was used. The dosage was one gramme given thrice the first day, two grammes thrice on the two succeeding days, and once on the morning of the fourth day—a total of 17 grammes.

1. *Subjective Sensations.*—These were absent, except in the case of the student, who either misunderstood, or disregarded instructions and took the salt in an unduly concentrated form, and, in consequence, experienced an increased sense of thirst. No increase of appetite was noticed.

2. *Observations on Blood Pressure.*—These were made for a few

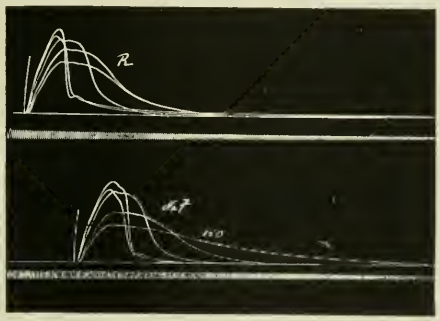

Fig. 4.—Fatigue curves. In the upper trace the muscle was bathed in Ringer's solution ; in the lower, in Ringer's solution containing (1 in 1000) sodium formate. Each 150th curve was recorded. Note the more increased latent period and relaxation period in the formate preparation.

days before, during, and after the course of sodium formate, by means of the Riva-Rocci instrument, the reading being taken when the pulse became imperceptible at the wrist. The variations gave no indication of action on the part of the salt:—

	Day before formate taken.	Fourth day.
F. W.	100	114
J. G. S.	100	90
I. M.	110	98
A. G.	110	114

3. *Ergographic Records.*—Mosso's instrument was used. A weight of 4·5 kilos. was raised over a pulley by flexion of the terminal phalanx of the right middle finger every two seconds until complete fatigue occurred.

The time was marked by a metronome beating seconds. Three of the subjects of experiment naturally sustained the weight until the intermediate second struck, the fourth relaxed the muscle immediately after the pull. We thought it well not to attempt to alter this arrangement, and have made no attempt to place the records on an equal basis, as each only applies to the individual in question. It is particularly difficult to get satisfactory ergographic records for purposes of comparison. Training undoubtedly, and suggestion in all probability, play an important part. In order to eliminate the effect of training two of the subjects practised with the ergograph daily until no further improvement occurred. The effects were remarkable. Unfortunately the early records in one case were not kept, but in the other there was an increase to eight times the original capacity for work in three weeks.

In the other two cases a single record was taken immediately before the formate course was begun, and a second record was taken four days later, when stiffness and pain on flexing the arm had disappeared. This method is probably not satisfactory, and it is interesting to note that after the formate course the untrained individuals showed improvement, the trained individuals showed diminution of work capacity.

Results:—

	Before.	After 2nd day.	After 4th day.	After 5th day.	After 6th day.	
F. W. (trained)	2·28	2·0	1·78	1·19	1·79	kilogrammetres
I. M. (trained).	12·68		10·19	...	...	„
A. G. . .	4·02		4·41	...	...	„
J. G. S. (contractions not sustained) .	10·23		21·92	...	...	„

Dynamometer Records.—The observations were made with the right arm held at right angles to the body.

	Before Formate.	After.
F. W.	30	29 kilograms.
J. G. S. . . .	34	33 „
I. M.	30	30 „
A. G.	57	55 „

These results all tend to show that in healthy human subjects sodium formate, in the doses given, has no appreciable effect.

Summary.

Formic acid, formate of potassium, sodium and calcium in minimal active doses exert a toxic action on the circulatory and muscular systems. This action is enormously added to by the effect of the metallic ion in the case of the potash salt, and may be obscured by the metallic ion in the case of the calcium salt.

With the exception of calcium formate in strong solution, they all lower blood pressure. They all have a transient vaso-dilator, followed by a vaso-constrictor, action.

They are all toxic to the heart and to skeletal muscle in the order potassium, sodium, calcium, and accelerate rather than retard fatigue.

In the dosage stated sodium formate has no demonstrable effect on the circulatory or muscular systems, or other noticeable effect on the healthy human subject.

Have the formates a place in therapeutics?

In so far as our experiments afford an answer to this question (assuming that the formates are absorbed) we should say (1) that the medicinal use of potassium formate would be unjustifiable; (2) that the effect of sodium formate might seriously impair the strength of a patient already debilitated; (3) that in calcium formate we have a lime salt, freely soluble, and not too unpalatable, which has an action in coagulating the blood both in virtue of its metallic and basic ion.

We venture to suggest that in cases of remote or intractable hæmorrhage it is worthy of a trial.

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A CLINICAL METHOD FOR ESTIMATING THE COAGULATION TIME OF THE BLOOD.

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In applying the method to be described the following apparatus are required :

Capillary glass tubes of certain dimensions.
A pricker.
A file or glass knife.
A flame for fusing the capillary tube.
A thermometer.

In case of accurate scientific determination, a chamber regulated to the temperature required for the observation

The bore of the capillary tubes is 1.5 mm., is uniform throughout, and the length of the tubes is about 6 in. The tubes are blown by a skilled glass-blower to as near the above dimensions as possible, and cost 2s. per 100. The other requisites require no explanation.

To Test the Coagulation-rate.

The hands are thoroughly washed with soap and water, and then held below the tap to wash off the last traces of soap. They are then dried on a clean towel. The minute from which the coagulation time is to date is then noted down, as well as the temperature of the room. With the watch marking seconds close at hand, the observer waits till the seconds hand is about ten seconds from the minute noted down. A rag is then bound tightly round the finger, and when the seconds hand reaches the exact minute, the finger is given a good sharp stab with the pricker. The capillary tube is then seized and applied to the drop, inclining it or keeping it horizontal according to the flow of blood, thus maintaining a continuous column in the capillary tube. About 4 in. of the tube can easily be filled in 10 seconds from the moment of pricking. The column of blood is then allowed to run down the tube to such a distance that it will not be injured by applying heat to the end into which the blood was received. This

injection and never once got opalescence, shows that intravenous injection is not a special cause. With regard to the explanation that

end is then fused (alternatively it may be closed with sealing wax or paraffin). The fusion keeps the column of blood steady, and care must be taken not to seize the tube between the sealed end and the end of the column of blood nearest it, otherwise the expansion of the air causes a movement of the column. The tube is then laid down with the thermometer beside it, and at the end of each minute a file mark is made on the capillary tube ½ in. or less from that end of the column of blood remote from the sealed end of the tube. This piece of the tube is then broken off, care being taken in breaking it to keep the two ends of the glass approximated. The two ends of the glass are then drawn apart, and the first stage of coagulation visible to the naked eye is shown by a minute thread of fibrin stretching between the two broken ends. When this is reached the other parts of the tube are similarly broken to confirm the first observation. A further confirmation can be got by holding up the capillary tube to the light and noticing that when coagulation has reached the stage of the formation of the thread, the column of blood in the tube, instead of having the regular contour it had before, has a ragged, irregular, whitish outline.

Points to be noted are that the hands must be clean and free from soap. The pricker must be cleaned after each prick with clean water; the finger must be similarly treated, and blood must not be drawn twice from the same prick. These latter precautions are necessary to avoid sucking up formed fibrin ferment into the tube with the blood and thus increasing the coagulation-rate. The only other point is with regard to the breaking of the tube. The tube must be broken and yet the ends kept together, otherwise the fibrin thread may be broken. One can suspect that this has happened by the blood column bulging out at the broken ends, as contrasted with the almost concave appearance one gets when the blood is fluid. If the thread has been broken, another piece should immediately be broken from the tube, a proceeding also undertaken, as noted above, for corroboration when the fibrin thread has been got.

I have done a good number of series of consecutive coagulation tests on myself. These were done in the evening. The time for the first appearance of the fibrin thread in all of them ranged from 7 to 9 minutes, the bulk of the readings being at 8 minutes. Of these series I shall give one, consisting of 8 consecutive readings done in my rooms from 8.3 p.m. to 9.18 p.m. (1¼ hours):

My finger and the pricker were washed in a tumbler of water and dried on a clean rag, and the capillary tube was fused in an ordinary fishtail burner. The temperature of the room ranged from 16.5° C. at the beginning to 17.5° C. at the end of the observations. Coagulation times in this series: First = 7 minutes; second = 8 minutes; third = 7 minutes; fourth = 9 minutes; fifth = 8 minutes; sixth = 8 minutes; seventh = 8 minutes; eighth = 8 minutes. Four other normal individuals gave at the same time the following readings: No. 1 = 6 minutes; 2 and 3 = 7 minutes; 4 = 8 minutes (twice).

I next give readings taken in the morning (9 a.m. to 11 a.m.) of 5 normal individuals with myself as control. The temperature of the room was 15° C.:

A = 10 minutes; B = 9 minutes; C = 11 minutes; D = 11 minutes; E = 11 minutes; myself = 11 minutes.

It will be seen that the coagulation-rate is much slower in the morning than in the evening, and Bürker, confirmed by Buckmaster, states that according to the time of day, amount of exercise, or kind of food, the coagulation-rate may be as low as 5 minutes or as high as 14.5.

The object of this note has been to demonstrate the possibilities of this method for clinical purposes. For clinical purposes, the providing of an apparatus for keeping the blood at a uniform temperature seems to be superfluous, as from all my readings done at room temperature, which varied from 15° C. to 20° C., there was no change in the coagulation-rate corresponding to elevation or lowering of temperature within these limits. This might be expected, considering that the blood is drawn at 37° C., that glass is a non-conductor of heat, and that the coagulation time is so short. It might be a factor to be considered in the case of bloods of long clotting time. In such cases a control with the observer's blood could be done and the result expressed as an index. This control would also correct any error from differences in roughness of the inner surface of the glass tubes in different samples of glass.

This method can also be used for the determination of the time of complete coagulation by breaking pieces off until, on pulling the two ends apart, the whole column of blood is pulled out from the broken-off piece. The separation of the serum from the clot can also be observed in these tubes and the differences in time of separation noted.

[illegible] always used intravenous injection and never once got opalescence, shows that intravenous injection is not a special cause. With regard to the explanation that

TURBIDITY OF THE SERUM IN RABBITS IN RELATION TO THE FORMATION OF AN ANTI-SERUM FOR THE PRECIPITIN TEST.

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Uhlenhuth (1902 [1]), Nuttall (1904 [2]), and others observed that the serum of rabbits used for preparing a precipitating serum in many cases was either opalescent or definitely milky; and one of the canons laid down by them for the preparation of a serum for use in medico-legal cases is that it shall not show any degree of opalescence. To keep up to this standard involves some slight difficulty and disappointment; for occasionally a rabbit may be brought up to a very high degree of precipitating power with its serum clear at the time the sample is taken for estimating its titre, and in the interval between the estimation and the killing of the rabbit to preserve its serum for future use (a few days) the serum for some unknown cause may become opalescen tor milky. Some workers think that it is due to the animal being killed at some stage of digestion, and empirically suggest that the animal be starved for a certain time,—usually for twenty-four hours before killing. The theory apparently at the back of this procedure is that the opalescence and turbidity is of the same nature as a lipæmic condition that may obtain in human beings, dogs, etc., after a meal. Another view as to the causation of the opalescence is that it is directly due to the rabbit being injected with the antigen—more especially if the antigen is injected intravenously. As regards the condition being due to the injection of the antigen, the fact that it occurs in rabbits which have never been injected and that it occurs extremely rarely in animals that have been injected (personally I have never seen it in preparing a series of about forty antisera in rabbits), show that this is not the case: and further, the fact that in preparing these forty antisera I always used intravenous injection and never once got opalescence, shows that intravenous injection is not a special cause. With regard to the explanation that

the phenomenon is due to feeding, Nuttall mentions that, although he bled his rabbits at all times without regard to feeding-time, he very rarely observed an opalescent serum. In this connection I might also mention that although I have had experience of thousands of bleedings of rabbits at all times of the day and at all times relative to the taking of food—the most of the rabbits being bled an hour or two after their morning feed—I have similarly had very few cases indeed of opalescent serum. So much has this been the case, that within the last eight months, although I have done hundreds of rabbits, and although I particularly desired to observe a rabbit with a good turbid serum, I have been unable to obtain one. Such has also been the experience of Dr. W. M. Scott in this laboratory, whose work involves taking samples of blood very extensively from rabbits. The phenomenon then must be due to something else in the metabolism of the rabbit than those changes concerned with the intake of food from the intestine, and which consequently could not be averted by starving the animal. As a matter of fact, starving the animal does not remove the turbidity when one gets a case of this kind. Moreover, the diet of rabbits—in contradistinction to that of man, dogs, etc., (which contains a large amount of fatty material)—consists mainly of carbohydrates, and contains very little fat. These facts should at the outset prevent a too hasty argument from analogy as to the cause of the condition in rabbits. Even if the substance causing the opalescence and milkiness is fat, which I believe to be the case—(although, owing to my not having obtained a good case[1] since I became interested in this subject, I have been unable to prove this definitely), there is no necessity to suppose that the fat must come from the intestine. For at the beginning of this year, while working at the effects of extensive bleedings on the complement content of rabbits' serum, I observed that even two days after one large bleeding (40 c.c.), the serum, which at this first bleeding was quite clear and free from opalescence, became opalescent; and with further bleedings became more and more so, until with extensive bleedings lasting daily over a week or so it became frankly milky. By the Adams modification of the Soxhlet extraction method I found that this milkiness coincided with a marked increase in the amount of fat in the serum—amounting to 1 per cent., while in the normal rabbit it is only about 0·2 to 0·4 per cent. Boggs and Morris (1909[4]) have observed the same occurrence, and to a much greater degree than I did, because they practised more extensive bleedings,—the amount of fat in the serum in some cases rising to about 4·5 per cent. They raise the question as to whether or not lipæmia has been observed often in

[1] I.e. one in which the serum was milky and in which the amount of fat would be much in excess of any error introduced in its estimation. The amount of fat present normally in rabbits' serum varies from about 0·2 to 0·4 per cent., and in the experimental lipæmias I found that more opalescence hardly altered that figure.

rapid anæmias in the human subject, and quote a case where they observed a moderate lipæmia in a case of secondary anæmia from hæmorrhoids. Dr. James Ritchie, Superintendent of this Laboratory, tells me of a case investigated by him some years ago, in which a terminal lipæmia occurred in a lad æt. 17, who died of an aplastic anæmia. Here, from about 20 c.c. of blood expressed from the inferior vena cava at the post-mortem, nearly 1 grm. of fat was recovered.

I was interested in this method of producing lipæmia in rabbits, because, as far as I know, it infallibly produces in rabbits a condition of serum similar to that which interferes so much with precipitin work. Unfortunately, as I have mentioned above, I could not determine whether the opalescence occurring normally is due to the same lipæmic condition, owing to my being unable to get a case sufficiently marked for accurate estimation.

As regards the experimental lipæmia in rabbits, one might hazard the view that it is due to the fat stores in the body being rapidly drawn on to make up for the drain of food material through the excessive bleeding. In this connection Boggs and Morris note that after the rabbit became extremely emaciated the milkiness of the serum gradually diminished. This might be due to all the fat stores of the body being used up. In the early stages of the experiment there would be the further factor of the fat in the bone marrow being rapidly removed to make room for the proliferation of the red marrow which takes place to cope with the acute anæmia that is produced. A rapid anæmia, which might conceivably involve a similar proliferation of the red marrow, occurs also with the pyridin poisoning used by Boggs and Morris. But with pyridin poisoning there is the additional factor of the actual poisonous nature of the substance which, by acting obscurely on the general metabolism of the rabbit, might prevent lipæmia, as Boggs and Morris found. If the opalescence occurring normally and sporadically in rabbits is due to fat, it might be explained by a similar mechanism to the above, of obscure origin and transient in nature, drawing suddenly on the fat stores of the body or on the blood forming capacity.

Whatever the cause is the condition is usually only temporary, for the opalescence disappears after a time. I have noticed, however, that it occurred more frequently and lasted longer in rabbits in poor condition, emaciated and generally very badly infected with tape-worm cysts intraperitoneally. Graham Smith (1904[3]) also found this.

Guinea-pigs very commonly have this turbid serum, and some human beings have it at all times irrespective of relation to meals. It is not caused by lactation in rabbits, for lactating does may be without it, and bucks have it as well as does.

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